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NEWS 2		"Ask CAS" for self-help around the clock
NEWS 3	Feb 24	PCTGEN now available on STN
NEWS 4	Feb 24	TEMA now available on STN
NEWS 5	Feb 26	NTIS now allows simultaneous left and right truncation
NEWS 6	Feb 26	PCTFULL now contains images
NEWS 7	Mar 04	SDI PACKAGE for monthly delivery of multifile SDI results
NEWS 8	Mar 24	PATDPAFULL now available on STN
NEWS 9	Mar 24	Additional information for trade-named substances without structures available in REGISTRY
NEWS 10	Apr 11	Display formats in DGENE enhanced
NEWS 11	Apr 14	MEDLINE Reload
NEWS 12	Apr 17	Polymer searching in REGISTRY enhanced
NEWS 13	Jun 13	Indexing from 1947 to 1956 added to records in CA/CAPLUS
NEWS 14	Apr 21	New current-awareness alert (SDI) frequency in WPIDS/WPINDEX/WPIX
NEWS 15	Apr 28	RDISCLOSURE now available on STN
NEWS 16	May 05	Pharmacokinetic information and systematic chemical names added to PHAR
NEWS 17	May 15	MEDLINE file segment of TOXCENTER reloaded
NEWS 18	May 15	Supporter information for ENCOMPPAT and ENCOMPLIT updated
NEWS 19	May 19	Simultaneous left and right truncation added to WSCA
NEWS 20	May 19	RAPRA enhanced with new search field, simultaneous left and right truncation
NEWS 21	Jun 06	Simultaneous left and right truncation added to CBNB
NEWS 22	Jun 06	PASCAL enhanced with additional data
NEWS 23	Jun 20	2003 edition of the FSTA Thesaurus is now available
NEWS 24	Jun 25	HSDB has been reloaded
NEWS 25	Jul 16	Data from 1960-1976 added to RDISCLOSURE
NEWS 26	Jul 21	Identification of STN records implemented
NEWS 27	Jul 21	Polymer class term count added to REGISTRY
NEWS 28	Jul 22	INPADOC: Basic index (/BI) enhanced; Simultaneous Left and Right Truncation available
NEWS 29	AUG 05	New pricing for EUROPATFULL and PCTFULL effective August 1, 2003
NEWS 30	AUG 13	Field Availability (/FA) field enhanced in BEILSTEIN

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NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
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=> s p53 and (intron or exon)
L1        8689 P53 AND (INTRON OR EXON)

=> s l1 and junction
L2         749 L1 AND JUNCTION

=> s l2 and probe
L3         545 L2 AND PROBE

=> s l3 and py<2001
   3 FILES SEARCHED...
L4         157 L3 AND PY<2001

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5          157 DUP REM L4 (0 DUPLICATES REMOVED)

=> s p53 and ((intron or exon)(p)junction (p) probe))
UNMATCHED RIGHT PARENTHESIS 'PROBE))'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s p53 and ((intron or exon)(p)junction (p) probe)
L6          22 P53 AND ((INTRON OR EXON)(P) JUNCTION (P) PROBE)

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7           22 DUP REM L6 (0 DUPLICATES REMOVED)
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=> d l7 ibib abs tot

L7 ANSWER 1 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2003:187821 USPATFULL
TITLE: Dual resonance energy transfer nucleic acid probes
INVENTOR(S): Bao, Gang, Mableton, GA, UNITED STATES
Tsourkas, Andrew, Atlanta, GA, UNITED STATES
Xu, Yangqing, Atlanta, GA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003129611	A1	20030710	
APPLICATION INFO.:	US 2002-179730	A1	20020625	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2001-300672P	20010625	(60)
	US 2001-303258P	20010703	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SUTHERLAND ASBILL & BRENNAN LLP, 999 PEACHTREE STREET, N.E., ATLANTA, GA, 30309
NUMBER OF CLAIMS: 50
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Page(s)
LINE COUNT: 2429
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Dual nucleic acid probes with resonance energy transfer moieties are provided. In particular, fluorescent or luminescent resonance energy transfer moieties are provided on hairpin stem-loop molecular beacon probes that hybridize sufficiently near each other on a subject nucleic acid, e.g. mRNA, to generate an observable interaction. The invention also provides lanthanide chelate luminescent resonance energy transfer moieties on linear and stem-loop probes that hybridize sufficiently near each other on a subject nucleic acid to generate an observable interaction. The invention thereby provides detectable signals for rapid, specific and sensitive hybridization determination in vivo. The probes are used in methods of detection of nucleic acid target hybridization for the identification and quantification of tissue and cell-specific gene expression levels, including response to external stimuli, such as drug candidates, and genetic variations associated with disease, such as cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 2 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2003:180711 USPATFULL
TITLE: Interventions to mimic the effects of calorie restriction
INVENTOR(S): Spindler, Stephen R., Riverside, CA, UNITED STATES
PATENT ASSIGNEE(S): The Regents of the University of California (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003124540	A1	20030703	
APPLICATION INFO.:	US 2002-56749	A1	20020122	(10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2000-648642, filed on 25 Aug 2000, GRANTED, Pat. No. US 6406853
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: TOWNSEND AND TOWNSEND AND CREW, LLP, TWO EMBARCADERO CENTER, EIGHTH FLOOR, SAN FRANCISCO, CA, 94111-3834

NUMBER OF CLAIMS: 28
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 13 Drawing Page(s)
LINE COUNT: 2446
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Long term calorie restriction has the benefit of increasing life span. Methods to screen interventions that mimic the effects of calorie restriction are disclosed. Extensive analysis of genes for which expression is statistically different between control and calorie restricted animals has demonstrated that specific genes are preferentially expressed during calorie restriction. Screening for interventions which produce the same expression profile will provide interventions that increase life span. In a further aspect, it has been discovered that test animals on a calorie restricted diet for a relatively short time have a similar gene expression profile to test animals which have been on a long term calorie restricted diet.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 3 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2003:174583 USPATFULL
TITLE: METHOD, SYSTEM AND COMPUTER SOFTWARE FOR ONLINE ORDERING OF CUSTOM PROBE ARRAYS
INVENTOR(S): Zhou , Xue Mei, 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
Smith , David P., 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
Kerr , Elizabeth M., 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
McLean , Lianne, 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
Sun , Shaw, 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
Siani-Rose , Michael A., 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
Mittman , Michael A., 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
Becker , Shawn H., 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
Jacobek , Lee A., 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
PATENT ASSIGNEE(S): Affymetrix, Inc., Santa Clara, 95051, UNITED STATES, California (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003120432	A1	20030626
APPLICATION INFO.:	US 2002-65868	A1	20021126 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2002-10063559, filed on 2 May 2002, Pending Continuation-in-part of Ser. No. WO 2002-US13902, filed on 2 May 2002, Pending

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-60265103	20010129

	US 2001-60301298	20010625
	US 2001-60306033	20010716
	US 2001-60333522	20011127
	US 2001-60343511	20011221
	US 2002-60349546	20020118
	US 2002-60375875	20020425
	US 2002-60376003	20020426
	US 2002-60394574	20020709
	US 2002-60403381	20020814

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
NUMBER OF CLAIMS: 84
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 17 Drawing Page(s)
LINE COUNT: 3497
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Abstract of Disclosure

A genomic portal system is described that receives user-selected identifiers of potential probes. The system determines verified probes corresponding to the identifiers and generates a custom probe array design. The system then displays the custom probe array design to the user via a graphical user interface and receives a user selection specifying acceptance, modification, or rejection of the design. The system provides the user with the accepted or modified custom probe array. The system may also enable a number of users to share space on a custom probe array. Another optional feature is to enable a number of users to share in ordering portions of a lot of catalog probe arrays to take advantage of economies of scale from lot-size purchases.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 4 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2003:152768 USPATFULL
TITLE: Nucleic acid detection methods using universal priming
INVENTOR(S): Fan, Jian-Bing, San Diego, CA, UNITED STATES
Fu, Xiang-Dong, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003104434	A1	20030605
APPLICATION INFO.:	US 2002-215644	A1	20020809 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2001-779202, filed on 7 Feb 2001, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	WO 2001-US4055	20010207
	US 2000-180810P	20000207 (60)
	US 2000-234731P	20000922 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: DORSEY & WHITNEY LLP, INTELLECTUAL PROPERTY DEPARTMENT, 4 EMBARCADERO CENTER, SUITE 3400, SAN FRANCISCO, CA, 94111
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 18 Drawing Page(s)
LINE COUNT: 2785
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to providing sensitive and accurate assays for gene detection, genome-wide gene expression profiling and alternative splice monitoring with a minimum or absence of target-specific amplification.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 5 OF 22 USPATFULL on STN

ACCESSION NUMBER: 2003:147248 USPATFULL

TITLE: METHOD, SYSTEM AND COMPUTER SOFTWARE FOR VARIANT INFORMATION VIA A WEB PORTAL

INVENTOR(S): Loraine , Ann E., 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
Helt , Gregg A., 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
Siani-Rose , Michael A., 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051
Kulp , David C., 3380 Central Expressway , Attn: Legal Department, Santa Clara, California, UNITED STATES 95051

PATENT ASSIGNEE(S): Affymetrix, Inc., Santa Clara, 95051, UNITED STATES, California (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003100995	A1	20030529
APPLICATION INFO.:	US 2002-65856	A1	20021126 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2002-10063559, filed on 2 May 2002, Pending Continuation-in-part of Ser. No. WO 2002-US13902, filed on 2 May 2002, Pending

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-60306033	20010716
	US 2001-60333522	20011127
	US 2001-60343511	20011221
	US 2002-60349546	20020118
	US 2002-60375875	20020425
	US 2002-60376003	20020426
	US 2002-60394574	20020709
	US 2002-60403381	20020814

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
NUMBER OF CLAIMS: 45
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 17 Drawing Page(s)
LINE COUNT: 3320

AB Abstract of Disclosure

A genomic web portal is described that receives from a user over the Internet a selection of identifiers of probes for detecting biological molecules. The portal may also receive hybridization intensity values produced from biological probe array experiments. The portal determines alternative splice variants based on factors that may include the hybridization intensity values. The portal correlates alternative splice variants with annotation data and provides for the user a graphical representation of the alternative splice variants and the correlated annotation data. The selection of annotation data to be displayed may be based on a user selection of a genomic, primary-transcript, mRNA, or protein display type. The annotation data may include genomic sequence; presence or relative abundance of alternative splice variants; exon arrangement, content, or sequence; frequency of exon usage in alternative splice variants; RNA, gene, or protein identification, function, structure, or sequence; probe arrangement; and other data.

L7 ANSWER 6 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2003:140414 USPATFULL
TITLE: Methods and kits for analysis of chromosomal rearrangements associated with cancer
INVENTOR(S): Felix, Carolyn A., Ardmore, PA, UNITED STATES
Jones, Douglas H., Cedar Rapids, IA, UNITED STATES
Rappaport, Eric, Blackwood, NJ, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003096255	A1	20030522
APPLICATION INFO.:	US 2002-118783	A1	20020409 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1998-26033, filed on 19 Feb 1998, GRANTED, Pat. No. US 6368791

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-38624P	19970219 (60)
	US 1997-56938P	19970825 (60)
	US 1997-65911P	19971117 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: DANN DORFMAN HERRELL & SKILLMAN, SUITE 720, 1601 MARKET STREET, PHILADELPHIA, PA, 19103-2307
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 37 Drawing Page(s)
LINE COUNT: 4379
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to kits and methods for panhandle PCR amplification of a region of DNA having an unknown nucleotide sequence, wherein the region flanks a region of a cancer-associated gene having a known nucleotide sequence in a human patient. Amplification of an unknown region flanking a known region of a cancer-associated gene permits identification of a translocation partner of the gene or identification of a replicated sequence within the gene. The invention further relates to kits useful for performing the methods of the invention, to an isolated polynucleotide, and to primers derived from such an isolated polynucleotide.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 7 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2003:106233 USPATFULL
TITLE: Compositions and methods for the therapy and diagnosis of pancreatic cancer
INVENTOR(S): Benson, Darin R., Seattle, WA, UNITED STATES
Kalos, Michael D., Seattle, WA, UNITED STATES
Lodes, Michael J., Seattle, WA, UNITED STATES
Persing, David H., Redmond, WA, UNITED STATES
Hepler, William T., Seattle, WA, UNITED STATES
Jiang, Yuqiu, Kent, WA, UNITED STATES
PATENT ASSIGNEE(S): Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003073144	A1	20030417
APPLICATION INFO.:	US 2002-60036	A1	20020130 (10)

	NUMBER	DATE

PRIORITY INFORMATION: US 2001-333626P 20011127 (60)
US 2001-305484P 20010712 (60)
US 2001-265305P 20010130 (60)
US 2001-267568P 20010209 (60)
US 2001-313999P 20010820 (60)
US 2001-291631P 20010516 (60)
US 2001-287112P 20010428 (60)
US 2001-278651P 20010321 (60)
US 2001-265682P 20010131 (60)
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
LINE COUNT: 14253
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compositions and methods for the therapy and diagnosis of cancer, particularly pancreatic cancer, are disclosed. Illustrative compositions comprise one or more pancreatic tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly pancreatic cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 8 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2003:44706 USPATFULL
TITLE: Detection of nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions
INVENTOR(S): Barany, Francis, New York, NY, UNITED STATES
Lubin, Matthew, Rye Brook, NY, UNITED STATES
Belgrader, Phillip, Manteca, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003032016	A1	20030213
APPLICATION INFO.:	US 2001-918156	A1	20010730 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1999-440523, filed on 15 Nov 1999, PATENTED Division of Ser. No. US 1997-864473, filed on 28 May 1997, PATENTED

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-18532P	19960529 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Michael L. Goldman, NIXON PEABODY LLP, Clinton Square, P.O. Box 31051, Rochester, NY, 14603
NUMBER OF CLAIMS: 54
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 29 Drawing Page(s)
LINE COUNT: 4257
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to the detection of nucleic acid sequence differences using coupled ligase detection reaction and polymerase chain reaction. One aspect of the present invention involves use of a ligase detection reaction coupled to a polymerase chain reaction. Another aspect of the present invention relates to the use of a primary polymerase chain reaction coupled to a secondary polymerase chain reaction coupled to a ligase detection reaction. A third aspect of the present invention involves a primary polymerase chain reaction coupled

to a secondary polymerase chain reaction. Such coupling of the ligase detection reaction and the polymerase chain reaction permits multiplex detection of nucleic acid sequence differences.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 9 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2003:30328 USPATFULL
TITLE: HUMAN NK-3 RELATED PROSTATE SPECIFIC GENE-1
INVENTOR(S): HE, WEI-WU, COLUMBIA, MD, UNITED STATES
CARTER, KENNETH C., NORTH POTOMAC, MD, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003022275	A1	20030130
APPLICATION INFO.:	US 1998-105470	A1	19980626 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-51080P	19970627 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: STERNE KESSLER GOLSTEIN & FOX, SUITE 600, 1100 NEW YORK AVENUE NW, WASHINGTON, DC, 200053934
NUMBER OF CLAIMS: 25
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Page(s)
LINE COUNT: 3630
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a novel member of the NK family of homeobox genes. In particular, isolated nucleic acid molecules are provided encoding the human NK-3 prostate specific gene 1 (NKX3.1) protein. NKX3.1 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of NKX3.1 activity. Also provided are diagnostic methods for detecting prostate cancer and other cancers and therapeutic methods for prostate cancer and other cancers.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 10 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2003:13207 USPATFULL
TITLE: Detection of nucleic acid sequence differences using the ligase detection reaction with addressable arrays
INVENTOR(S): Barany, Francis, 450 E. 63rd St., Apt. #12C, New York, NY, United States 10021
Gerry, Norman P., 308 E. 83 St. 1C, New York, NY, United States 10028
Witowski, Nancy E., 7224 Tara Rd., Edina, MN, United States 55439
Day, Joseph, 1147 Chess Dr., Foster City, CA, United States 94404
Hammer, Robert P., 4967 Tulane Dr., Baton Rouge, LA, United States 70808
Barany, George, 1813 Prior Ave. N., Falcon Heights, MN, United States 55113

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6506594	B1	20030114
APPLICATION INFO.:	US 2000-526992		20000316 (9)

	NUMBER	DATE

PRIORITY INFORMATION:	US 1999-125357P	19990319 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	
PRIMARY EXAMINER:	Whisenant, Ethan C.	
ASSISTANT EXAMINER:	Lu, Frank W	
LEGAL REPRESENTATIVE:	Nixon Peabody LLP	
NUMBER OF CLAIMS:	75	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	88 Drawing Figure(s); 46 Drawing Page(s)	
LINE COUNT:	5007	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention describes a method for identifying one or more of a plurality of sequences differing by one or more single base changes, insertions, deletions, or translocations in a plurality of target nucleotide sequences. The ligation phase utilizes a ligation detection reaction between one oligonucleotide probe, which has a target sequence-specific portion and an addressable array-specific portion, and a second oligonucleotide probe, having a target sequence-specific portion and a detectable label. After the ligation phase, the capture phase is carried out by hybridizing the ligated oligonucleotide probes to a solid support with an array of immobilized capture oligonucleotides at least some of which are complementary to the addressable array-specific portion. Following completion of the capture phase, a detection phase is carried out to detect the labels of ligated oligonucleotide probes hybridized to the solid support. The ligation phase can be preceded by an amplification process. The present invention also relates to a kit for practicing this method, a method of forming arrays on solid supports, and the supports themselves.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 11 OF 22 USPATFULL on STN

ACCESSION NUMBER:	2002:251077 USPATFULL
TITLE:	Spliced gene of KSHV / HHV8, its promoter and monoclonal antibodies specific for LANA2
INVENTOR(S):	Chang, Yuan, Irvington, NY, UNITED STATES Moore, Patrick S., Irvington, NY, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002137020	A1	20020926	
APPLICATION INFO.:	US 2000-733728	A1	20001208	(9)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	APPLICATION			
LEGAL REPRESENTATIVE:	John P. White, Cooper & Dunham, LLP, 1185 Avenue of the Americas, New York, NY, 10036			
NUMBER OF CLAIMS:	94			
EXEMPLARY CLAIM:	1			
NUMBER OF DRAWINGS:	14 Drawing Page(s)			
LINE COUNT:	2177			

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides an isolated nucleic acid which encodes a Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen 2 polypeptide (LANA2) or a fragment thereof and also provides the LANA2 polypeptide. This invention provides an isolated nucleic acid comprising consecutive nucleotides having the sequence of a promoter of Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen 2 transcription. This invention also provides a method of inhibiting **p53** mediated apoptosis of a cell and a method of producing an antibody which comprises introducing into a cell a replicable vector of the subject invention.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 12 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2002:193026 USPATFULL
TITLE: METHOD FOR IDENTIFYING ALZHEIMER'S DISEASE THERAPEUTICS USING TRANSGENIC ANIMAL MODELS
INVENTOR(S): GAMES, KATE DORA, BELMONT, CA, UNITED STATES
SCHENK, DALE BERNARD, BURLINGAME, CA, UNITED STATES
MCCONLOGUE, LISA CLAIRE, SAN FRANCISCO, CA, UNITED STATES
SEUBERT, PETER ANDREW, SAN FRANCISCO, CA, UNITED STATES
RYDEL, RUSSELL E., BELMONT, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002104104	A1	20020801
APPLICATION INFO.:	US 1998-149718	A1	19980908 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1996-660487, filed on 7 Jun 1996, ABANDONED Continuation-in-part of Ser. No. US 1995-480653, filed on 7 Jun 1995, ABANDONED Continuation-in-part of Ser. No. US 1996-659797, filed on 7 Jun 1996, ABANDONED Continuation-in-part of Ser. No. US 1995-486538, filed on 7 Jun 1995, ABANDONED
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: TOWNSEND AND TOWNSEND AND CREW, LLP, TWO EMBARCADERO CENTER, EIGHTH FLOOR, SAN FRANCISCO, CA, 94111-3834
NUMBER OF CLAIMS: 27
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Page(s)
LINE COUNT: 4514
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The construction of transgenic animal models of human Alzheimer's disease, and methods of using the models to screen potential Alzheimer's disease therapeutics, are described. The models are characterized by pathologies similar to pathologies observed in Alzheimer's disease, based on expression of all three forms of the .beta.-amyloid precursor protein (APP), APP695, APP751, and APP770, as well as various point mutations based on naturally occurring mutations, such as the London and Indiana familial Alzheimer's disease (FAD) mutations at amino acid 717, predicted mutations in the APP gene, and truncated forms of APP that contain the A.beta. region. Animal cells can be isolated from the transgenic animals or prepared using the same constructs with standard techniques such as lipofection or electroporation. The transgenic animals, or animal cells, are used to screen for compounds altering the pathological course of Alzheimer's disease as measured by their effect on the amount of APP, .beta.-amyloid peptide, and numerous other Alzheimer's disease markers in the animals, the neuropathology of the animals, as well as by behavioral alterations in the animals.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 13 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2002:144075 USPATFULL
TITLE: Interventions to mimic the effects of calorie restriction
INVENTOR(S): Spindler, Stephen R., Riverside, CA, United States
PATENT ASSIGNEE(S): The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6406853	B1	20020618
APPLICATION INFO.:	US 2000-648642		20000825 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1999-471225, filed

on 23 Dec 1999
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Jones, W. Gary
ASSISTANT EXAMINER: Taylor, Janell E.
LEGAL REPRESENTATIVE: Townsend & Townsend & Crew LLP
NUMBER OF CLAIMS: 26
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 13 Drawing Figure(s); 13 Drawing Page(s)
LINE COUNT: 2230
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Long term calorie restriction has the benefit of increasing life span. Methods to screen interventions that mimic the effects of calorie restriction are disclosed. Extensive analysis of genes for which expression is statistically different between control and calorie restricted animals has demonstrated that specific genes are preferentially expressed during calorie restriction. Screening for interventions which produce the same expression profile will provide interventions that increase life span. In a further aspect, it has been discovered that test animals on a calorie restricted diet for a relatively short time have a similar gene expression profile to test animals which have been on a long term calorie restricted diet.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 14 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2001:123406 USPATFULL
TITLE: Nucleic acid marker for cancer
INVENTOR(S): Ware, Joy L., Richmond, VA, United States
Dechsukhum, Chavaboon, Richmond, VA, United States
Garrett, Carleton T., Richmond, VA, United States

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2001010914	A1	20010802
APPLICATION INFO.:	US 2001-756910	A1	20010110 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1999-434620, filed on 5 Nov 1999, GRANTED, Pat. No. US 6232073

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-118749P	19990205 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: McGuire Woods, Suite 1800, 1750 Tysons Boulevard, McLean, VA, 22102
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Page(s)
LINE COUNT: 899
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides using a truncated WT1 gene transcript as a marker for detecting cancer in a subject. The method provides detecting the truncated WT1 gene transcript in a sample from the subject where the truncated gene transcript is characterized by an absence of a 101 base pair segment of intron 5 between nucleic acid positions -101 and -1. Positive detection of the truncated WT1 gene transcript indicates the presence of cancer. The invention provides a truncated WT1 gene transcript characterized by an absence of a 101 base pair segment of intron 5 between nucleic acid positions -101 and -1 and having a length of about two thousand base pairs. The truncated gene transcript is further characterized by containing at their five prime end sequences normally confined to the fifth intron of the WT1 gene, exons six through ten at their three prime end, and an overall length of approximately 2

kb.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 15 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2001:121255 USPATFULL
TITLE: Detection of nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions
INVENTOR(S): Barany, Francis, 450 E. 63rd St., New York, NY, United States 10021
Lubin, Matthew, 20 Magnolia Dr., Rye Brook, NY, United States 10573-1820
Belgrader, Phillip, 719 Pebble Way, Manteca, CA, United States 95336

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6268148	B1	20010731
APPLICATION INFO.:	US 1999-440523		19991115 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1997-864473, filed on 28 May 1997, now patented, Pat. No. US 6027889

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-18532P	19960529 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Horlick, Kenneth R.
LEGAL REPRESENTATIVE: Nixon Peabody LLP
NUMBER OF CLAIMS: 26
EXEMPLARY CLAIM: 23
NUMBER OF DRAWINGS: 45 Drawing Figure(s); 29 Drawing Page(s)
LINE COUNT: 3653
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to the detection of nucleic acid sequence differences using coupled ligase detection reaction and polymerase chain reaction. One aspect of the present invention involves use of a ligase detection reaction coupled to a polymerase chain reaction. Another aspect of the present invention relates to the use of a primary polymerase chain reaction coupled to a secondary polymerase chain reaction coupled to a ligase detection reaction. A third aspect of the present invention involves a primary polymerase chain reaction coupled to a secondary polymerase chain reaction. Such coupling of the ligase detection reaction and the polymerase chain reaction permits multiplex detection of nucleic acid sequence differences.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 16 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2001:71308 USPATFULL
TITLE: Nucleic acid marker for cancer
INVENTOR(S): Ware, Joy L., Richmond, VA, United States
Dechsukhum, Chavaboon, Richmond, VA, United States
Garrett, Carleton T., Richmond, VA, United States
PATENT ASSIGNEE(S): Virginia Commonwealth University, Richmond, VA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6232073	B1	20010515
APPLICATION INFO.:	US 1999-434620		19991105 (9)

	NUMBER	DATE

PRIORITY INFORMATION: US 1999-118749P 19990205 (60)
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Myers, Carla J.
LEGAL REPRESENTATIVE: McGuireWoods, LLP
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Figure(s); 11 Drawing Page(s)
LINE COUNT: 845
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides using a truncated WT1 gene transcript as a marker for detecting cancer in a subject. The method provides detecting the truncated WT1 gene transcript in a sample from the subject where the truncated gene transcript is characterized by an absence of a 101 base pair segment of intron 5 between nucleic acid positions -101 and -1. Positive detection of the truncated WT1 gene transcript indicates the presence of cancer. The invention provides a truncated WT1 gene transcript characterized by an absence of a 101 base pair segment of intron 5 between nucleic acid positions -101 and -1 and having a length of about two thousand base pairs. The truncated gene transcript is further characterized by containing at their five prime end sequences normally confined to the fifth intron of the WT1 gene, exons six through ten at their three prime end, and an overall length of approximately 2 kb.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 17 OF 22 USPATFULL on STN
ACCESSION NUMBER: 2000:21383 USPATFULL
TITLE: Detection of nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions
INVENTOR(S): Barany, Francis, New York, NY, United States
Lubin, Matthew, Rye Brook, NY, United States
PATENT ASSIGNEE(S): Cornell Research Foundation, Inc., Ithaca, NY, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6027889		20000222	
APPLICATION INFO.:	US 1997-864473		19970528	(8)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-18532P	19960529 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Horlick, Kenneth R.
LEGAL REPRESENTATIVE: Nixon, Hargrave, Devans & Doyle LLP
NUMBER OF CLAIMS: 28
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 45 Drawing Figure(s); 29 Drawing Page(s)
LINE COUNT: 4414
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to the detection of nucleic acid sequence differences using coupled ligase detection reaction and polymerase chain reaction. One aspect of the present invention involves use of a ligase detection reaction coupled to a polymerase chain reaction. Another aspect of the present invention relates to the use of a primary polymerase chain reaction coupled to a secondary polymerase chain reaction coupled to a ligase detection reaction. A third aspect of the present invention involves a primary polymerase chain reaction coupled to a secondary polymerase chain reaction. Such coupling of the ligase detection reaction and the polymerase chain reaction permits multiplex detection of nucleic acid sequence differences.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 18 OF 22 USPATFULL on STN
ACCESSION NUMBER: 1999:27435 USPATFULL
TITLE: Nucleic acid molecules coding for tumor suppressor proteins and methods for their isolation
INVENTOR(S): Spengler, Dietmar, Munich, Germany, Federal Republic of
Journot, Laurent, Pignan, France
PATENT ASSIGNEE(S): Max-Planck-Gesellschaft zur Forderung der Wissenschaften e.V., Berlin, Germany, Federal Republic of (non-U.S. corporation)
CNRS, Montpellier, France (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5876972		19990302	
APPLICATION INFO.:	US 1996-718661		19960923	(8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Patterson, Jr., Charles L.
LEGAL REPRESENTATIVE: White, John P.Cooper & Dunham LLP
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 37 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 2193
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Described are novel proteins having the biological activity of a tumor suppressor protein and nucleic acid molecules coding for such proteins. Methods for the isolation of nucleic acid molecules encoding tumor suppressor proteins as well as nucleic acid molecules obtainable by said method are also provided. Further, vectors comprising said nucleic acid molecules wherein the nucleic acid molecules are operatively linked to regulatory elements allowing expression in prokaryotic or eukaryotic host cells can be used for the production of polypeptides encoded by said nucleic acid molecules which have tumor suppressor activity. Pharmaceutical and diagnostic compositions are provided comprising the nucleic acid molecules of the invention and/or comprising a nucleic acid molecule which is complementary to such a nucleic acid molecule. Described are also compositions which comprise polypeptides encoded by the described nucleic acid molecules which have tumor suppressor activity and/or an antibody specifically recognizing such polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 19 OF 22 USPATFULL on STN
ACCESSION NUMBER: 97:86431 USPATFULL
TITLE: Diagnostic test for the desmoplastic small round cell tumor
INVENTOR(S): Ladanyi, Marc, New York, NY, United States
Gerald, William, Pelham, NY, United States
PATENT ASSIGNEE(S): Sloan-Kettering Institute for Cancer Research, New York, NY, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5670317		19970923	
APPLICATION INFO.:	US 1995-437027		19950508	(8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Myers, Carla J.
LEGAL REPRESENTATIVE: White, John P.
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 15 Drawing Figure(s); 11 Drawing Page(s)
LINE COUNT: 1850
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides an isolated nucleic acid molecule encoding a chimeric EWS-WT1 protein. This invention also provides an isolated protein which is a chimeric EWS-WT1 protein. This invention further provides a method of diagnosing a desmoplastic small round cell tumor in a subject which comprises detecting in a sample from the subject a nucleic acid molecule encoding a chimeric EWS-WT1 protein, positive detection indicating the presence of desmoplastic small round cell tumor. This invention also provides a method of inhibiting the growth of a neoplastic cell, wherein the cell is characterized by the presence of a chimeric EWS-WT1 protein which comprises contacting an antibody which specifically recognizes the chimeric EWS-WT1 fusion protein under suitable conditions so that an antibody-antigen complex is formed, thereby inhibiting the growth of the neoplastic cell.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 20 OF 22 USPATFULL on STN
ACCESSION NUMBER: 97:9938 USPATFULL
TITLE: Human prohibitin DNA
INVENTOR(S): Nakamura, Yusuke, Tokyo, Japan
Sato, Takaaki, Tokyo, Japan
PATENT ASSIGNEE(S): Cancer Institute, Tokyo, Japan (non-U.S. corporation)
Eisai Co., Ltd., Tokyo, Japan (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5599707		19970204
APPLICATION INFO.:	US 1995-370789		19950110 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1993-114461, filed on 31 Aug 1993, now patented, Pat. No. US 5401635 which is a division of Ser. No. US 1993-9255, filed on 22 Jan 1993, now abandoned

	NUMBER	DATE
PRIORITY INFORMATION:	JP 1992-11156	19920124
	JP 1992-308654	19921118

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Horlick, Kenneth R.
LEGAL REPRESENTATIVE: Flynn, Thiel, Boutell & Tanis, P.C.
NUMBER OF CLAIMS: 2
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Figure(s); 3 Drawing Page(s)
LINE COUNT: 865
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A human prohibitin gene, a protein coded for by said gene, a gene analysis reagent to be used with them, and a quantitative determination of prohibitin in a biological sample by an immunological technique with the use of an antihuman prohibitin antibody and a method for analyzing a prohibitin gene of a human tissue for the occurrence of mutation by the PCR method with the use of oligonucleotides having partial base sequences of said gene as primers.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 21 OF 22 USPATFULL on STN
ACCESSION NUMBER: 95:97110 USPATFULL
TITLE: Anti-human prohibitin antibodies
INVENTOR(S): Nakamura, Yusuke, Tokyo, Japan
Sato, Takaaki, Tokyo, Japan

PATENT ASSIGNEE(S): Cancer Institute, Tokyo, Japan (non-U.S. corporation)
Eisai Co., Ltd., Tokyo, Japan (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5463026		19951031
APPLICATION INFO.:	US 1994-192156		19940204 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1993-114461, filed on 31 Aug 1993, now patented, Pat. No. US 5401635 which is a division of Ser. No. US 1993-9255, filed on 22 Jan 1993, now abandoned

	NUMBER	DATE
PRIORITY INFORMATION:	JP 1992-11156	19920124
	JP 1992-308654	19921118

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Lacey, David L.
ASSISTANT EXAMINER: Loring, Susan A.
LEGAL REPRESENTATIVE: Flynn, Thiel, Boutell, & Tanis
NUMBER OF CLAIMS: 1
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Figure(s); 3 Drawing Page(s)
LINE COUNT: 853
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An antibody which specifically binds with a human prohibitin or a partial structural fragment thereof can be used as a diagnostic agent in the detection of cancer. The human prohibitin has the structure illustrated in SEQ ID NO:1.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 22 OF 22 USPATFULL on STN
ACCESSION NUMBER: 95:27203 USPATFULL
TITLE: Nucleic acids encoding human prohibitin mutants and detection thereof
INVENTOR(S): Nakamura, Yusuke, Tokyo, Japan
Sato, Takaaki, Tokyo, Japan
PATENT ASSIGNEE(S): Cancer Institute, Tokyo, Japan (non-U.S. corporation)
Eisai Co., Ltd., Tokyo, Japan (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5401635		19950328
APPLICATION INFO.:	US 1993-114461		19930831 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1993-9255, filed on 22 Jan 1993

	NUMBER	DATE
PRIORITY INFORMATION:	JP 1992-11156	19920124
	JP 1992-308654	19921118

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Parr, Margaret
ASSISTANT EXAMINER: Horlick, Kenneth R.
LEGAL REPRESENTATIVE: Flynn, Thiel, Boutell & Tanis
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 2
NUMBER OF DRAWINGS: 3 Drawing Figure(s); 3 Drawing Page(s)
LINE COUNT: 903
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A human prohibitin gene, a protein coded for by said gene, a gene analysis reagent to be used with them, and a quantitative determination

of prohibitin in a biological sample by an immunological technique with the use of an antihuman prohibitin antibody and a method for analyzing a prohibitin gene of a human tissue for the occurrence of mutation by the PCR method with the use of oligonucleotides having partial base sequences of said gene as primers.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d kwic tot

L7 ANSWER 1 OF 22 USPATFULL on STN

DETD . . . the art that are indicative of a disease state. The methods include the detection of nucleic acids comprising K-ras, survivin, **p53**, p16, DPC4, or BRCA2. Furthermore, the methods can be used to detect the amount of a subject nucleic acid being. . .

DETD . . . K-ras mutations can lead to early detection of pancreatic carcinomas. Other oncogenes and tumor-suppressor genes involved in pancreatic cancer include **p53**, p16, MADH4, DPC4, BRCA2, MKK4, STK11, TGFBR1 and TGFBR2.

DETD [0200] To further examine **probe**-target hybridization and energy transfer between nucleic acid probes of the present invention, dual-FRET molecular beacons were designed and synthesized. Specifically,. . . the target sequence. The loop portion, therefore, is 13 bases in length. The synthetic targets mimicking the GAPDH IVT RNA **exon** 6/**exon** 7 **junction** are designed so that the gap between the two beacons hybridizing on the same target is respectively 3, 4, 5. . .

CLM What is claimed:
48. The method of claim 43, wherein the subject nucleic acid comprises K-ras, survivin, **p53**, p16, DPC4, or BRCA2.

L7 ANSWER 2 OF 22 USPATFULL on STN

DETD [0072] A 223 base pair (bp) DNA fragment made up of 110 bases of **intron** 3 and all 113 bases of **exon** 4 of the mouse GRP78 gene was synthesized by PCR using genomic DNA as template and inserted into pT7/T3 (Ambion, Austin, Tex.). Two probes of the **junction** region of **intron** 7 and **exon** 7 of the GRP78 gene were produced by PCR using mouse genomic DNA as template. A 257-base fragment including all of **exon** 7 and the first 113 bases of **intron** 7 was produced. A 200-base fragment including all of **exon** 7 and the first 56 bases of **intron** 7 also was produced. The T7 RNA polymerase promoter was ligated to these PCR fragments using a Lig'nScribe kit as. . . assays were performed using an RPA II kit as described by the supplier (Ambion). Hybridization of the 257 base RNA **probe** with GRP78 pre-mRNA protected all 257-bases corresponding to **exon** 7 and the first 113 bases of **intron** 7. Hybridization of the 200-base RNA **probe** to pre-mRNA protected 200 bases corresponding to all of **exon** 7 and the first 56 bases of **intron** 7. Hybridization of either **probe** to GRP78 mRNA protects the 143-bases complementary to **exon** 7. A 185- and a 277-bp cDNA fragment of S-II cDNA was synthesized and subcloned into pT7/T3 (12). [.sup.32P]-labeled RNA. . .

DETD . . . Caspase 3 (Casp3); cysteine protease mediator of apoptosis; ubiquitous; ET63241

3 4 0.005 Cyclin G (Ccng); augments apoptosis; target gene of **p53**; liver, elsewhere; Z37110

>100 >100 <0.001 Fused toes (Fts); a gene related to ubiquitin-conjugating enzymes; suggested role in apoptosis during development;

expression distribution poorly defined; X71978
22 21 <0.001 **P53** specific ubiquitin ligase 2 (Mdm2); promotes
ubiquitination and proteaesome degradation of **p53**;
inactivation by stress causes cell cycle arrest and apoptosis;
liver, elsewhere; X58876
>100 >100 <0.001 RNA-dependent EIF-2 alpha kinase; double-stranded RNA-
. . .

L7 ANSWER 3 OF 22 USPATFULL on STN
DETD . . . number of genes or EST's are known to be involved with this biological process. For example, a gene known as **p53** is involved with tumor suppression, and this information is stored in one or more of the databases accessible from database. . . provides to user 101 a list of probe-set identifiers that includes the one or more probe-set identifiers associated with gene **p53**. The list of probe-set identifiers may be provided to the user in one of numerous possible formats. For example, the. . .
DETD [0100] In a preferred embodiment, **probe** sets are designed to identify specific alternative splice variants. For example, a **probe** set may consist of probes designed to interrogate the exons of a particular alternative splice variant as well as **junction** probes designed to interrogate the region where two specific exons are predicted to be joined together. The **junction probe** may interrogate, for instance, the sequence of the 3" end of **exon** 1 and the 5" end of **exon** 3. In the present example, an alternative splice variant mRNA that comprises exons 1 and 3 will hybridize to the **exon** probes and, if the splice variant is joined in the correct orientation, it will also hybridize to the one or more **junction** probes. Additional examples of alternative splice variant **probe** sets and **probe** arrays are described in U.S. Patent Application Serial No. 09/697,877, titled "METHODS FOR MONITORING THE EXPRESSION OF ALTERNATIVLEY SPLICED GENES",. . . Provisional Patent Application Serial No. 60/362,524, titled "METHODS FOR DETERMINING A MINIMAL SET OF PROBES FOR ALTERNAITVE SPLICING NUCLEIC ACID **PROBE** ARRAY DESIGN", filed March 6, 2002; each of which is hereby incorporated by reference herein in its entirety for all. . .
DETD . . . PRT Plus Array, HuGeneFL Array, Human Genome U95 Set, Human Genome U133 Set, HuSNP Probe Array, Murine Genome U74 Set, **P53** Probe Array, Rat Genome U34 Set, Rat Neurobiology U34 Set, Rat Toxicology U34 Array, Human Genome Focus Array, or Yeast. . .
DETD . . . translation and site 1327 may represent the site of termination of transcription and/or translation. Also displayed in pane 1325 is **exon probe** set sites 1340 and **junction** **probe** set sites 1345 that are illustrative examples of **probe** set annotations. Sites 1340 represent the regions of exons that are interrogated by **probe** sets, and similarly sites 1345 displays the relationship of **probe** sets that interrogate the **junction** region where two exons may be spliced together. In the illustrated implementation, each of the displayed boxes of sites 1340 may represent a single **probe** set whereas each of the displayed boxes of sites 1345 may represent a portion of a **probe** set that may, for instance, include a box representing half a **probe** set that interrogates the sequence region at the end of one **exon** (e.g., the 5" end) and another box representing the remaining half of the **probe** set may interrogate the sequence at the end of another **exon** (e.g., the 3" end). In some implementations it is not necessary that adjacent boxes of sites 1345 belong to the same **probe** set, rather each box may be representative of some portion of a **probe** set that may be used

in combination with a box belonging to sites 1345 representing a complementary portion. For example, a box belonging to sites 1345 at the 5" end of **exon** one may represent a portion of a **probe** set that could, for instance, be half the number of probes of a **probe** set. A complimentary box could be located at the 3" end of **exon** two, three, or the 3" end of any **exon** contained within a particular gene that contains the remaining portion of a **probe** set that identifies a splice variant containing **exon** one spliced to **exon** two, three, or other **exon** defined by the **probe** set.

L7 ANSWER 4 OF 22 USPATFULL on STN

DETD . . . can take on a wide variety of conformations, depending on the assay. For example, when expression profiling or alternate splice **junction** analysis is to be performed, a single target **probe** can be used. Thus, a single **probe** can be designed for any mRNA sequence, with an upstream and downstream universal primer. After separation of the hybridization complexes and amplification, the detection of the mRNA sequence proceeds as outlined below. In the case of splice **junction** analysis, the target specific portion of the **probe** has a first domain that hybridizes to the first **exon** and a second domain that hybridizes to the second **exon**, and the assay is run under conditions whereby only if both domains hybridize to the target mRNA does the hybridization. . .

DETD [0036] Alternatively, in a preferred embodiment, for example in alternate splice **junction** analysis, two probes can be used; in this embodiment, the oligonucleotide ligation assay (OLA) can be performed. OLA relies on. . . the termini, i.e. at a detection position. In this embodiment, there are two ligation probes: a first or upstream ligation **probe** that comprises the upstream universal priming sequence and a second portion that will hybridize to a first domain of the target mRNA sequence (e.g. the terminus of a first **exon**, which is therefore a splice **junction** specific **probe**), and a second or downstream ligation **probe** that comprises a portion that will hybridize to a second domain of the target mRNA sequence (e.g. complementary to the terminus of a second **exon**), adjacent to the first domain, and a second portion comprising the downstream universal priming sequence. If perfect complementarity at the **junction** exists, the ligation occurs and then the resulting hybridization complex (comprising the mRNA target and the ligated **probe**) can be separated as above from unreacted probes. Again, the universal priming sites are used to amplify the ligated **probe** to form a plurality of amplicons that are then detected in a variety of ways, as outlined herein.

DETD . . . the protein level. p16 is known to control cell cycle through the Rb pathway whereas p14ARF is involved in the **p53** pathway. Both RASL and RT-PCR yielded similar expression profiles of the two isoforms in cell lines 1, 3, 4, and. . .

L7 ANSWER 5 OF 22 USPATFULL on STN

SUMM . . . include any one, or any combination of, the following data: genomic sequence; presence and/or relative abundance of alternative splice variants; **exon** arrangement, content, and/or sequence; **intron** arrangement, content, and/or sequence; frequency of **exon** usage in two or more of the alternative splice variants; isoform identification; primary transcript, mRNA or other RNA identification, function,. . . protein-based annotations of the coding regions; start and stop codons; 5' transcriptional control elements; 3' polyadenylation signals; splice site boundaries; **probe** arrangement, content, and/or sequence; and/or expression level data corresponding to one or more probes of the **probe** sets. In various implementations, the probes may be constructed and arranged to detect mRNA expression. Also, the probes may include

exon probes and/or **junction** probes.

DETD . . . number of genes or EST's are known to be involved with this biological process. For example, a gene known as **p53** is involved with tumor suppression, and this information is stored in one or more of the databases accessible from database. . . provides to user 101 a list of probe-set identifiers that includes the one or more probe-set identifiers associated with gene **p53**. The list of probe-set identifiers may be provided to the user in one of numerous possible formats. For example, the. . .

DETD [0091] In a preferred embodiment, **probe** sets are designed to identify specific alternative splice variants. For example, a **probe** set may consist of probes designed to interrogate the exons of a particular alternative splice variant as well as **junction** probes designed to interrogate the region where two specific exons are predicted to be joined together. The **junction probe** may interrogate, for instance, the sequence of the 3" end of **exon** 1 and the 5" end of **exon** 3. In the present example, an alternative splice variant mRNA that comprises exons 1 and 3 will hybridize to the **exon** probes and, if the splice variant is joined in the correct orientation, it will also hybridize to the one or more **junction** probes. Additional examples of alternative splice variant **probe** sets and **probe** arrays are described in U.S. Patent Application Serial No. 09/697,877, titled "METHODS FOR MONITORING THE EXPRESSION OF ALTERNATIVLEY SPLICED GENES",. . . Provisional Patent Application Serial No. 60/362,524, titled "METHODS FOR DETERMINING A MINIMAL SET OF PROBES FOR ALTERNAITVE SPLICING NUCLEIC ACID **PROBE** ARRAY DESIGN", filed March 6, 2002; each of which is hereby incorporated by reference herein in its entirety for all. . .

DETD . . . PRT Plus Array, HuGeneFL Array, Human Genome U95 Set, Human Genome U133 Set, HuSNP Probe Array, Murine Genome U74 Set, **P53** Probe Array, Rat Genome U34 Set, Rat Neurobiology U34 Set, Rat Toxicology U34 Array, Human Genome Focus Array, or Yeast. . .

DETD . . . translation and site 1327 may represent the site of termination of transcription and/or translation. Also displayed in pane 1325 is **exon probe** set sites 1340 and **junction probe** set sites 1345 that are illustrative examples of **probe** set annotations. Sites 1340 represent the regions of exons that are interrogated by **probe** sets, and similarly sites 1345 displays the relationship of **probe** sets that interrogate the **junction** region where two exons may be spliced together. In the illustrated implementation, each of the displayed boxes of sites 1340 may represent a single **probe** set whereas each of the displayed boxes of sites 1345 may represent a portion of a **probe** set that may, for instance, include a box representing half a **probe** set that interrogates the sequence region at the end of one **exon** (e.g., the 5" end) and another box representing the remaining half of the **probe** set may interrogate the sequence at the end of another **exon** (e.g., the 3" end). In some implementations it is not necessary that adjacent boxes of sites 1345 belong to the same **probe** set, rather each box may be representative of some portion of a **probe** set that may be used in combination with a box belonging to sites 1345 representing a complementary portion. For example, a box belonging to sites 1345 at the 5" end of **exon** one may represent a portion of a **probe** set that could, for instance, be half the number of probes of a **probe** set. A complimentary box could be located at the 3" end of **exon** two, three, or the 3" end of any **exon** contained within a particular gene that contains the remaining portion of a **probe** set that identifies a splice variant containing **exon** one spliced to **exon** two, three, or other **exon** defined by the **probe** set.

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DRWD . . . MLL bcr rearrangements in ALL of patient 38 identified by (A) Southern blot analysis of BamHI-digested DNA with B859 cDNA **probe** (Felix et al., 1997, Blood 90: 4679-4686; Felix et al., 1998, J Pediatr Hematol/Oncol. 20: 299-308) (arrows, left panel) and. . . the 7.0 kb fragment was from MLL-AF-4 rearrangement (Felix et al., 1997, Blood 90: 4679-4686). (B) Sequence of genomic breakpoint **junction** of other derivative chromosomes in recombination-PCR generated subclones derived by reverse panhandle PCR. 35 bp of 5' sequence are from. . . through ligated oligonucleotide (P-Oligo). 1028-1030 bp of 5' sequence are CDK6. The 3' 1176-1178 bp include MLL bcr sequence from **intron** 9 through nested MLL primer 3. Arrowheads show CDK6 and MLL breakpoint positions; `AG` nucleotide sequence in both genes precluded. . . sequences are shown (middle). (C) Detection of CDK6-MLL fusion transcript. RT-PCR reactions with primers from CDK6 exons 1-2 and MLL **exon** 13, and randomly primed cDNA template produced a 548 bp product (top). Reactions using .beta.-actin primers and RNA-negative reagent control (dH.sub.2O) are shown (top). Sequencing revealed in-frame fusion of CDK6 **exon** 2 at position 486 of the 1249 bp CDK6 cDNA (GenBank accession no. NM.sub.--001259) to MLL **exon** 10 (bottom). (D) cdk6 and MLL proteins and predicted cdk6-MLL fusion protein.

DETD . . . GGA CA-3' (SEQ ID NO: 43). from CDK6 intron 2, to determine if a reciprocal AF-4-CDK6 rearrangement had occurred, and **p53** exon 8 primers were used in a positive control reaction (Felix et al., 1998, Blood 91: 4451-4456).

L7 ANSWER 7 OF 22 USPATFULL on STN

SUMM [2043] SEQ ID NO:2003 is **the** determined cDNA sequence of clone 61496359

DETD . . . (384 384 96 Clone ID Ratio Signal 1 Signal 2 Blastn

75 PCX352_r01c15 a 15 838:A8 80150 2.37 0.648 0.273 Hu. **p53**-induced protein PIGPC1, transmembrane protein (THW gene)

76 PCX352_r01c16 a 16 838:B8.sup. 80151 1.88 0.292 0.156 Hu. serine (or cysteine) proteinase inhibitor,. . . tumor suppressor (Drosophila) homolog (FAT)

80 PCX352_r04c11 d 11 838:G6 80155 1.55 0.999 0.645 Hu. mRNA for transmembrane protein (THW gene), **p53**-induced protein PIGPC1 (PIGPC1)

81 PCX352_r07c03 g 3 .sup. 839:E2 80156 0.95 0.253 0.266 Hu. fibrillarin (FBL)

82 PCX352_r08c06 h 6 839:H3 80157 0.76 0.273 0.359 Hu. fibrillarin (FBL)

83 PCX352_r10c16 j 16 840:D8 80158 2.75 0.937 0.34 Hu. **p53**-induced protein PIGPC1, transmembrane protein (THW gene)

84 PCX352_r10c24 j 24 840:D12 80159 5.14 3.94 0.767 Hu. similar to collagen, type I,. . . 0.071 0.027 Hu. highly similar to glucose-6-phosphate dehydrogenase; ubiquitin-like protein (GdX)

92 PCX353_r02c11 b 11 842:C6.sup. 80168 2.87 1.156 0.403 Hu. **p53**-induced protein PIGPC1, transmembrane protein (THW gene)

93 PCX353_r02c23 b 23 .sup. 842:C12 80169 2.72 1.001 0.368 Hu. **p53**-induced protein PIGPC1, transmembrane protein (THW gene)

94 PCX353_r06c08 f 8 843:D4 80170 1.84 1.602 0.87 Hu. keratin 18 (KRT18)

95 PCX353_r07c22 g. . . 80180 1.57 0.095 0.06 Hu.

small EDRK-rich factor 1B
(centromeric) (SERF1B)
101 PCX354_r03c02 c 2 846:F1.sup. 80181 1.8 0.951
0.53 Hu. **p53**-induced protein PIGPC1,
transmembrane protein (THW gene)
102 PCX354_r04c04 d 4 846:H2 80183 1.17 0.301 0.258
Hu. fibrillarin (FBL)
103 PCX354_r04c10 d 10. . . h 23 847:G12 80189 2.38
1.421 0.596 Hu. tumor antigen (L6)
109 PCX354_r09c15 i 15 848:A8 80191 2.44 0.602 0.247
Hu. **p53**-induced protein PIGPC1,
transmembrane protein (THW gene)
110 PCX354_r09c19 i 19 848:A10 80192 2.22 0.753 0.339
Hu. **p53**-induced protein PIGPC1,
transmembrane protein (THW gene)
111 PCX354_r11c13 k 13 .sup. 848:E7 80193 2 0.391
0.196 Hu. sema domain, immunoglobulin
domain. . . 848:E10 80194 2.2 1.718 0.783 Hu.
connective tissue growth factor
(CTGF)
113 PCX354_r12c15 l 15 848:G8 80195 2.15 1.144 0.533
Hu. **p53**-induced protein PIGPC1,
transmembrane protein (THW gene)
114 PCX355_r01c03 a 3 850:A2 80196 1.79 0.092 0.051
Human mitochondrion
115 PCX355_r02c11 b 11 850:C6.sup.. . . 845:H8 80179
1.76 1.259 0.715 Hu. transmembrane protein (THW gene),
PIGPC1
122 PCX354_r09c03 i 3 848:A2 80190 2.35 0.789 0.336
Hu. **p53**-induced protein PIGPC1,
transmembrane protein (THW g
123 PCX355_r11c11 k 11 .sup. 852:E6 80200 1.9 0.881
0.463 Hu. tumor antigen (L6)
124 PCX355_r15c13. . .
DETD . . . sequences for Pancreas cDNAs
SEQ ID NO:
(Full-Length cDNA/Pro) CLONE NAME CLONE ID GENBANK IDENTITY/SEQUENCE FROM BLASTN OF SEQID

130/153 IodesPancChip2-1 80150 Hu. **p53**-induced protein PICPC1, transmembrane protein (THW gene)
131/154 IodesPancChip2-2 80151 Hu. serine (or cysteine) proteinase inhibitor, clade E
132/155 IodesPancChip2-3 81052 Hu. keratin. . .

L7 ANSWER 8 OF 22 USPATFULL on STN
SUMM . . . Microsatellite markers have also been used for colon cancer detection (L. Cawkwell, et. al., "Frequency of Allele Loss of DCC, **p53**, RB1, WT1, NF1, NM23, and APC/MCC in Colorectal Cancer Assayed by Fluorescent Multiplex Polymerase Chain Reaction," Br. J. Cancer 70(5):. . .
DRWD [0094] FIGS. 26A-C show electropherogram results for an LDR/PCR process of ErbB, G6PD, Int2, **p53**, and SOD gene segments from normal human female DNA and from DNA of the breast cancer cell line ZR-75-30 and. . . FAM-labeled products of 58 bp (ErbB (i.e. HER-2/neu/erbB oncogene)), 61 bp (G6PD), 67 bp (Int2 (i.e. int-2 oncogene)), 70 bp (**p53**) and 76 bp (SOD). These products are separated and analyzed on a 373A DNA sequencer using the Genescan 672 software. . . determination for the five loci in normal human female DNA is shown. The peak heights and areas for G6PD, Int2, **p53**, and SOD are very similar. The peak height and area for ErbB is consistently small in normal genomic DNA. In. . .
DRWD . . . of ErbB affected the relative peak heights of the other LDR

oligonucleotide probes and PCR oligonucleotide primers for G6PD, Int2, **p53**, and SOD. In FIG. 27A, the gene dosage determination for the four loci in normal human female DNA is shown. Peak heights and areas for G6PD, Int2, **p53**, and SOD are similar, as they were in the experiment using all five LDR primers. In FIG. 27B, G6PD, Int2,. . . cancer cell line show similar relative peak heights, comparable to their appearance in normal female DNA. The peak height for **p53** is reduced, suggesting the deletion of this gene in a portion of the cells in this cell line. In FIG. 27C, in the gastric carcinoma cell line, SKGT-2, G6PD, and **p53** show comparable peak heights. The Int2 peak height remains relatively high, as it was in the experiment using all five. . .

DETD . . . of 104, 107, and 110, with the peak areas representing amplification of the Her-2 gene, loss of heterozygosity of the **p53** gene, and the control SOD gene, respectively. The electrophoresis curve where steps 3b and 4b are used involves three ligation. . . of 58, 70, and 76, with the peak areas representing amplification of the Her-2 gene, loss of heterozygosity of the **p53** gene, and the control SOD gene, respectively.

DETD . . . sequences hybridizing to gene-specific addresses, where the fluorescent intensity represents amplification of the Her-2 gene, loss of heterozygosity of the **p53** gene, and the control SOD gene, respectively.

DETD . . . process for multiplex detection of gene amplifications and deletions. Here, the ratio of the Her-2/neu gene from Chromosome 17q, the **p53** gene from Chromosome 17p, and the SOD gene from Chromosome 21q is detected. Following denaturation of DNA at 94.degree. C.,. . .

DETD . . . both alleles (i.e., chromosomes) containing RB1 and NM23 and loss of heterozygosity (i.e., loss of allele on one chromosome) for **p53**.

DETD . . . both alleles (i.e., chromosomes) containing RB1 and NM23 and loss of heterozygosity (i.e., loss of allele on one chromosome) for **p53**.

DETD . . . oncogenes, tumor suppressor genes, or genes involved in DNA amplification, replication, recombination, or repair. Examples of these include: BRCA1 gene, **p53** gene, APC gene, Her2/Neu amplification, Bcr/Ab1, K-ras gene, and human papillomavirus Types 16 and 18. Various aspects of the present. . .

DETD . . . erbBEx1-6R (48) exon "1" P40

G6PD Xq28 G6PDEx6-3L (48) G6PDEx6-4R (48) exon 6 W1145

Int2 11q13 Int2Ex3-7L (50) Int2Ex3-8R (46) exon 3 W135

p53 17p13.1 p53Ex8-9L (52) p53Ex8-10R (44) exon 8 P51

SOD 21q22.1 SODEx3-11L (49) SODEx3-12R (47) exon 3 P355

DETD . . . oligonucleotide probes for quantification of gene amplifications and deletions in the LDR/PCR process. These oligonucleotide probes were designed to recognize **exon** 8 in the **p53** tumor suppressor gene (on chromosome 17p), **exon** 3 of int-2 (on chromosome 11q), an internal **exon** in HER-2/neu (i.e. HER-2/neu/erbB oncogene) (on chromosome 17q), **exon** 3 in SOD (i.e. super oxide dimutase) (on chromosome 21q), and **exon** 6 in G6PD (i.e. glucose 6-phosphate dehydrogenase) (on chromosome Xq). Each pair of LDR oligonucleotide probes has the following features: (i) The left oligonucleotide **probe** contains from 5' to 3' an 18 base sequence identical to the fluorescently labeled secondary oligonucleotide primer (black bar), an. . . target-specific sequence of from 22 to 28 bases with a T.sub.m of 75.degree. C. (patterned bar); (ii) The right oligonucleotide **probe** contains from 5' to 3' a target-specific sequence of 20-25 bases with a T.sub.m of 75.degree. C. (patterned bar), a. . . each

unique restriction site generates a product which differs by at least 2 bases from the other products. Each oligonucleotide **probe** set has an **exon**-specific region chosen to ligate the **junction** sequence of (A, T)C.dwnarw.C(A, T). This **junction** sequence corresponds to either a proline residue (codon CCN) or the complementary sequence of a tryptophan residue (TGG). These sequences were chosen to minimize differences in ligation rates and the chance of a polymorphism at the ligation **junction**.

DETD [0233] In the normal female, the ErbB2 peak is lower, and the **p53** peak is slightly lower than the remaining 3 peaks. See FIGS. 25A-D. In different experiments, it was observed that the ErbB2 peak is always lower; the G6PD, Int-2, **p53**, and SOD peak areas would vary somewhat, but all 5 peaks would retain the same relative profile from one sample. . . these two cell lines. In addition, cell line NM10 appears to have undergone LOH (i.e. a loss of heterozygosity) of **p53**, while cell line SKBR3 appears to have undergone LOH of G6PD and **p53**. Some of the cells in cell line SKBR3 may have lost both copies of the **p53** gene. Repeating these amplifications in the absence of the ErbB-2 primers was used to confirm the presence of these additional. . .

DETD . . . The raw data and ratio of peak areas are given below:

TABLE 3

Raw Peak Area Data

	Genes ErbB	G6PD	Int2	**p53**	SOD
Male	9954	21525	45688	36346	62506
Female	8340	39309	39344	30270	54665
NM10	20096	55483	67083	17364	84339
SKBR3	106650	19120	50103	2888. . .	

DETD [0235]

TABLE 4

Ratio of Peak Areas to SOD Peak Area

	ErbB/SOD	G6PD/SOD	Int2/SOD	**p53**/SOD
Male	0.16	0.34	0.73	0.58
Female	0.15	0.72	0.72	0.55
NM10	0.24	0.66	0.80	0.21
SKBR3	2.22	0.40	1.04	0.06

DETD . . . of peak area ratios between normal DNA and cancer cell lines.

TABLE 5

Ratio of Peak Areas Ratios

	ErbB/2	G6PD	Int2	**p53**
Female/Male	0.96	2.09	0.98	0.95
NM10/Male	1.50	1.91	1.09	0.35
SKBR3/Male	13.92	1.15	1.42	0.10

DETD . . . can be determined that the normal male and female have the same number of genes on chromosomes 17q (ErbB), 17p (**p53**), and 11q (Int 2), but that the female has twice as many G6PD genes, or X chromosomes. Likewise, cell line NM10 showed slight amplification of the ErbB-2 gene, and LOH at **p53**, while cell line SKBR3 shows significant amplification of the ErbB-2 gene, LOH at G6PD and **p53**. To confirm additional gene amplifications and deletions, primer pairs causing massive amplifications may be removed from the LDR/PCR reaction (see. . .

DETD . . . the ErbB2 peak is lower than the remaining 4 peaks. In different experiments, it was observed that the G6PD, Int-2, **p53**

, and SOD peak areas would vary somewhat, but would retain the same relative profile from one sample to the next.. . . the known ErbB-2 gene amplification in these two cell lines. In addition, the ZR-75-30 line appears to show LOH of **p53**, while the SKGT-2 cell line appears to have a slight amplification of the Int-2 region. By repeating these LDR/PCR experiments. . .

DETD . . . The raw data and ratio of peak areas are given below:

TABLE 6

Raw Peak Area Data

	Genes ErbB	G6PD	Int2	**p53**	SOD
Female; 4 Primer Sets	NA	9577	8581	9139	8128
ZR7530; 4 Primer Sets	NA	8452	7904	4168	7996
SKGT2; 4 Primer Sets. . .					

DETD [0240]

TABLE 7

Ratio of Peak Areas to SOD Peak Area

	ErbB/SOD	G6PD/SOD	Int2/SOD	**p53**/SOD
Female; 4 Primer Sets	NA	1.18	1.06	1.12
ZR7530; 4 Primer Sets	NA	1.06	0.99	0.52
SKGT2; 4 Primer Sets	NA	1.28	2.29. . .	

DETD [0242] One can quantify the amount of ErbB2 and Int-2 amplification as well as **p53** deletion by comparing the ratio of peak area ratios between normal DNA and cancer cell lines, as shown in Table. . . sets of primers to ascertain the internal consistency of this technique.

TABLE 8

Ratio of Peak Area Ratios

	ErbB	G6PD	Int2	**p53**
Female; 4/5	NA	1.10	1.16	1.07
ZR7530; 4/5	NA	0.89	1.04	1.16
SKGT2; 4/5	NA	0.79	0.97	1.04
ZR7530/Female; 4/4. . .				

DETD . . . and 5 primer amplifications (with the exception of SKGT2-G6PD noted above). The ZR7530 cell line demonstrates a clear LOH for **p53**, while the SKGT2 cell line shows amplification of the Int-2 region, and both **p53** genes present

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DETD . . . tumor suppressor genes, which encode transcription factors which suppress cell growth, such as the Rb gene for retinoblastoma or the **p53** gene in colon cancer (Huang et al., Science 242: 1563-1566 (1988); Barker, et al., Science 249: 912-915 (1980); toxic proteins. . .

DETD . . . (Shen, M. M. and Leder, P., Proc. Natl. Acad. Sci. USA 89:8240-8244 (1992)). The RNAse protection assays were performed using **probe** A which spans the **intron-exon** splice **junction**; similar results were obtained using probes B or C. Northern blot analysis was performed essentially as described (Ausubel, F. et. . .

L7 ANSWER 10 OF 22 USPATFULL on STN

DRWD . . . using an addressable array. FIG. 35A shows a schematic representation of LDR probes used to distinguish mutations. Each allele specific **probe** contains an addressable sequence complement (Z1

or Z3) on the 5'-end and the discriminating base on the 3'-end. The common LDR **probe** is phosphorylated on the 5'-end and contains a fluorescent label on the 3'-end. The probes hybridize adjacent to each other. . . DNA, and the nick will be sealed by the ligase if and only if there is perfect complementarity at the **junction**. FIG. 35B shows the presence and type of mutation is determined by hybridizing the contents of an LDR reaction to. . . of chromosomal DNA containing the K-ras gene. Exons are shaded and the position of codons 12 and 13 are shown. **Exon**-specific probes were used to selectively amplify K-ras DNA flanking codons 12 and 13. Probes were designed for LDR detection of. . .

DETD FIG. 1 depicts the detection of a germline point mutation, such as the **p53** mutations responsible for Li-Fraumeni syndrome. In step 1, after DNA sample preparation, exons 5-8 are PCR amplified using Taq (i.e.. . .

DETD FIG. 2 depicts detection of somatic cell mutations in the **p53** tumor suppressor gene but is general for all low sensitivity mutation detection. In step 1, DNA samples are prepared and. . .

DETD . . . oncogenes, tumor suppressor genes, or genes involved in DNA amplification, replication, recombination, or repair. Examples of these include: BRCA1 gene, **p53** gene, Familial polyposis coli, Her2/Neu amplification, Bcr/Ab1, K-ras gene, human papillomavirus Types 16 and 18, leukemia, colon cancer, breast cancer,. . .

DETD . . . on the addressable solid support array. The concept is shown in two possible formats, for example, for detection of the **p53** R248 mutation (FIGS. 13A-C).

DETD . . . alternative formats for oligonucleotide probe design to identify the presence of a germ line mutation in codon 248 of the **p53** tumor suppressor gene. The wild type sequence codes for arginine (R248), while the cancer mutation codes for tryptophan (R248W). The. . . used in a similar fashion. FIG. 13B shows two LDR probes that are designed to discriminate wild type and mutant **p53** by containing the discriminating base C or T at the 3' end. In the presence of the correct target DNA. . .

L7 ANSWER 11 OF 22 USPATFULL on STN

AB . . . of a promoter of Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen 2 transcription. This invention also provides a method of inhibiting **p53** mediated apoptosis of a cell and a method of producing an antibody which comprises introducing into a cell a replicable. . .

SUMM . . . viral genome to host chromatin during mitosis, effecting equal segregation of viral genome during division (3). LANA1 also binds to **p53** and inhibits **p53**-mediated transcriptional activity and apoptosis (13). vCYC over-expression induces apoptosis (31) and it is at least theoretically possible that this may. . .

SUMM . . . E1A proteins also activate cMYC but use differing sets of coadaptors from those used by vIRF1 (19). vIRF1 additionally inhibits **p53**- and Fas-induced apoptosis ((5) and unpublished obs, S. Jayachandra, P. S. Moore, Y. Chang). vIRF1, however, is not generally expressed. . . and having NF-kB-inhibitory activity has been described (6). We show here that LANA2 is a B-cell specific factor that antagonizes **p53** tumor suppressor functions and is expressed during latency.

SUMM . . . (ORFK10.5) appear to have arisen through gene duplication of a captured cellular IRF gene. LANA2 is a potent inhibitor of **p53** -induced transcription in reporter assays. LANA2 antagonizes apoptosis due to **p53** overexpression in **p53**-null SAOS-2 cells and apoptosis due to doxorubicin treatment of wild-type **p53** U20S cells. While LANA2 specifically interacts with aminoacids 290-393 of **p53** in glutathione-S-transferase pull-down assays, we were unable to demonstrate LANA2-**p53** interaction in vivo by immunoprecipitation. These findings show that KSHV has tissue-specific latent gene expression programs and identify a new latent protein which

may contribute to KSHV tumorigenesis in hematopoietic tissues via **p53** inhibition.

SUMM [0026] This invention provides a method of inhibiting **p53** mediated apoptosis of a cell which comprises introducing into the cell an effective amount of the replicable vector which comprises. . . nucleic acid which encodes Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen 2 polypeptide or fragment thereof, so as to thereby inhibit **p53** mediated apostosis of the cell.

SUMM . . . the isolated nucleic acid which encodes Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen 2 polypeptide or fragment thereof effective to inhibit **p53** mediated apoptosis of the cell, so as to thereby immortalize the cell.

SUMM . . . vector which comprises the isolated nucleic acid which encodes Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen 2 polypeptide effective to inhibit **p53** mediated apoptosis of the cell producing the antibody and thereby immortalizing the cell, so as to thereby produce the antibody.

DRWD [0054] Inhibition of **p53** transcriptional activity by LANA2. Representative luciferase assay showing inhibition of reporter gene expression by transient transfection of pcDNA.LANA2: A. SAOS-2 cells were transfected with 2 mg of plasmid pG13-Luc reporter plasmid together with 0.0 or 0.5 mg of pcDNA.**p53** and 0.5 or 1 mg pcDNA.LANA2 as indicated. For control, SAOS-2 cells were transfected with the reporter plasmid pGL3-control and. . .

DRWD [0056] In vitro GST pull down assays using [.sup.35S]methionine labeled LANA2 or **p53**. LANA2 interacts with full length **p53** protein as well as the **p53** region between 290-393 aa

DRWD [0058] LANA2 inhibits **p53**-induced apoptosis. SAOS-2 cells were transfected with pEGFP-F* and the empty expression vector pCDNA3.1 (A), pCDNA.**p53** (B) or pCDNA.**p53** and pCDNA.LANA2(C). Total DNA in all transfections was normalized using empty expression vector. After 48h, cells were fixed and stained. . .

DETD [0109] Studies have shown that LANA2 polypeptide can inhibit **p53** mediated apoptosis. Accordingly, this invention provides a method of inhibiting **p53** mediated apoptosis of a cell which comprises introducing into the cell an effective amount of the replicable vector which comprises. . . nucleic acid which encodes Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen 2 polypeptide or fragment thereof, so as to thereby inhibit **p53** mediated apostosis of the cell.

DETD . . . the isolated nucleic acid which encodes Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen 2 polypeptide or fragment thereof effective to inhibit **p53** mediated apoptosis of the cell, so as to thereby immortalize the cell.

DETD . . . herein, "immortalizing" refers to the action of LANA2 polypeptide in a B cell wherein the LANA2 polypeptide interacts with the **p53** mediated apoptosis pathway to inhibit the action of **p53** in the cell. The above interaction does not allow the cell to die, thereby creating an "immortalized" cell.

DETD . . . vector which comprises the isolated nucleic acid which encodes Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen 2 polypeptide effective to inhibit **p53** mediated apoptosis of the cell producing the antibody and thereby immortalizing the cell, so as to thereby produce the antibody.. . .

DETD . . . was verified on an ABI 377 Sequenator (Applied Bio-systems Inc., Foster City, Calif.) pG13-Luc, a reporter plasmid containing 13 tandem **p53**-response elements derived from the p21 promoter, was a gift from W. El-Deiry and B. Volgelstein (4). pGL-3 control (Promega, Madison, Wis.) was used as a control vector for luciferase transient transfection assays. GST-**p53**(full length (FL)) and the C-terminus fragment of **p53** (GST-**p53** (290-393)) plasmids were a gift from W. Gu (17). DNA sequences corresponding to the 1-100 and 100-290 aminoacids of human **p53** were amplified by PCR and subcloned into pGEX-KG (18) to generate the protein expression

plasmids GST-**p53** (1-100) and GST-**p53** (100-290).
pcDNA.**p53** expression plasmid was a gift of RT Hay (35).
pEGFP-F* (gift of W. Jiang) expresses green fluorescent protein (GFP) and was used as a marker for pcDNA.LANA2 and/or pcDNA.**p53** transfection to gate fluorescent cells by FACS. The plasmid containing the Gal-4 binding domain (Gal4-BD), PAS2-1, the Gal4-activation domain (Gal4-AD), pGAD424, as well as the plasmids containing the DNA-BD/murine **p53** fusion protein PVA3 and the DNA-AD/murine **p53** fusion protein pGADp53 and control plasmids pCL1, PLAM5', pGBT9 and pTD1 were obtained from Clontech (Clontech laboratories, Palo Alto, Calif.).

DETD . . . transfected plasmid for each experimental condition. Each measurement was performed in triplicate, with experiments independently replicated at least three times. **p53**-null SAOS-2 cells were co-transfected with 2 mg pG13-Luc in the presence or absence of 0.5 mg pcDNA.**p53** with or without pcDNA.LANA2 (0.5-1 mg). U20S cells were co-transfected with 2 mg pG13-Luc in the presence or absence of. .

DETD . . . 1.times.10.sup.6 SAOS-2 cells were transfected (Cell Phect) with 1 mg of the GFP expressing plasmid, pEGFP-F*, in the presence of pcDNA.**p53** (4.5 mg) and/or pcDNA.LANA2 (4.5 mg) or the empty expression vector. U20S cells were transfected with 1 mg pEGFP-F* in. .

DETD [0163] GST in vitro binding assays were performed using in vitro translated [S.sup.35] methionine-labeled LANA2 incubated with **p53** GST fusion proteins (GST-**p53** (FL), GST-**p53** (1-100), GST-**p53**(100-290), GST-**p53** (290-393), and GST alone. In vitro translated [S.sup.35] methionine-labeled **p53** was incubated with GST-LANA2 and GST alone.

DETD [0165] LANA2 (20 mg of pcDNA.LANA2) and **p53** (20 mg of pcDNA. **p53**) were expressed in SAOS-2 cells by co-transfection and were immunoprecipitated with anti-LANA2 CM-8B6 or CM-10A2 antibodies, or D0-1 (Santa Cruz Biotech, Santa Cruz, Calif.), Pab 1801 (Santa Cruz,), and Ab-1 (Oncogene, Cambridge, Mass.) anti-**p53** antibodies. Protein complexes were resolved by SDS/10% PAGE and transferred onto nitrocellulose membrane. LANA2 was detected using CM-8B6, CM-10A2 and **p53** was detected using D0-1, Pab 1801, Ab-1 by immunoblotting and enhanced chemiluminescence (ECL, Amersham, Piscataway, N.J.).

DETD . . . GAL4-AD in the plasmid pGAD424 or to GAL4 DNA-binding domain (BD) in the plasmid pAS2-1. The plasmids containing the murine **p53** fused to GAL4 AD or GAL4BD were provided by Clontech. The yeast strain Y-190 was used for this two hybrid. . .

DETD [0176] Since the transcript size identified by the V3 **probe** is incompatible with the predicted transcript for putative ORFK10.5, we screened a cDNA library made from TPA-stimulated BC-1 cells to. . . are present in the f703 insert, but only one of the five other phage inserts extended through the 5' splice **junction**. Splicing results in a 1704 bp full length transcript for the newly annotated gene which is designated ORFK10.5 to distinguish it from the unspliced 3' **exon** previously designated K10.1 (FIG. 2, GenBank Accession No. A4008303). This ORF is composed of a novel 455 bp 5' **exon** that is joined to the 3' **exon** 1339 bp internally to and out of frame with the previously annotated ORF K10.1 predicted from the genome sequence analysis. . .

DETD [0183] LANA2 Inhibits **p53** Transactivation

DETD [0184] Since LANA1 inhibits **p53**-mediated transcription and apoptosis (13), we examined the effects of LANA2 on **p53** function using the pG13-Luc promoter reporter (containing 13 copies of the **p53** response element) transiently transfected into SAOS-2 (**p53** null) osteosarcoma cells. Transient expression of 0.5 mg **p53** plasmid in SAOS-2 cells resulted in an 800-fold activation of the pG13-Luc reporter which was inhibited by 87% on cotransfection. . . activation was seen at low levels of LANA2 expression and increasing amounts of pcDNA.LANA2 resulted in a monotonic repression of **p53** activity on the pG13 reporter.

DETD [0185] To determine if the same effect is present during endogenous **p53** activation, these experiments were repeated in U20S cells (wild-type for **p53**) with and without treatment with 0.4 mM doxorubicin, a chemotherapeutic agent which induces **p53** -mediated apoptosis. Doxorubicin treatment resulted in 13-fold activation of the pG13-Luc reporter and this effect was inhibited 57% by 0.5 mg. . .

DETD [0187] To determine if inhibition of **p53** transactivation is due to direct interaction with **p53** protein, we performed full length and truncated GST-**p53** pulldown assays using in vitro translated [.sup.35S]-methionine-labeled LANA2. As seen in FIG. 9, GST-**p53** fusion protein precipitates LANA2 in vitro whereas no interaction is seen with GST protein alone. LANA2 interaction is localized to the region of **p53** comprising aa 290-393 and no interaction occurs with the truncated **p53** constructs containing aa 1-100 or aa100-290. In the reverse pull-down experiments, GST-LANA2 but not GST alone showed specific interaction with in vitro translated full length **p53**.

DETD [0188] In vivo coimmunoprecipitation experiments, however, failed to demonstrate direct interaction between LANA2 and **p53** (not shown). In experiments using naturally abundant **p53** from BCBL-1 cells or SAOS-2 cells in which **p53** protein was overexpressed, no coimmunoprecipitation was detected for LANA2 and **p53** using either LANA2 (CM-10A2 and CM-8B6) or **p53** (D0-1, Pab 1801, Ab-1) monoclonal antibodies. In part these experiments were inconclusive since we noted an unusual phenomenon in that D0-1 (Santa Cruz), Pab 1801 (Santa Cruz) and Ab-1 (Oncogene) antibodies directed against **p53** directly cross-react with LANA2. This was confirmed by direct western blotting with these antibodies and the bacteria-derived GST-LANA protein in the absence of **p53**. We thus cannot exclude artifactual **p53**-LANA2 interactions in the GST-pulldown assays, or that antibody binding occurs at LANA2-**p53** interaction site(s) which interfers with the immunoprecipitation reaction since the binding was done under native conditions. Yeast two-hybrid assays between LANA2 and full-length **p53** failed to clarify whether or not direct protein-protein interactions occur in vivo (data not shown). LANA2 cloned into the Gal4-BD cassette is toxic to the yeast and could not be evaluated. LANA2 cloned into the Gal4-AD cassette and **p53** into the Gal4-BD cassette, however, shows no interaction by b-galactosidase assay.

DETD [0189] LANA2 Inhibits **p53**-Mediated Apoptosis

DETD [0190] SAOS-2 cells are null for pRB as well as **p53**, and overexpression of wild-type **p53** in SAOS-2 cells results in apoptosis as indicated by the subdiploid fraction (20%) of cells staining with propidium iodide in a cell sorting profile (FIG. 10). In this experiment, cells were cotransfected with **p53** and GFP expression plasmids, and DNA content analysis was performed only on cells gated for GFP. When LANA2 is expressed together with **p53** in SAOS-2 cells (FIG. 10C), a marked diminution in subdiploid cells (from 20% to 10.8%) occurs indicating a specific inhibition of **p53**-mediated apoptosis and genomic fragmentation. Similar results are obtained for U20S cells, which have wild-type **p53**, treated with 0.4 uM doxorubicin for 30 hours, indicating that LANA2 can inhibit activation of endogenous **p53** resulting from doxorubicin treatment (FIG. 10F). This was confirmed by caspase-8 activation fluorometric assays. Doxorubicin treated U20S cells transfected with. . .

DETD . . . cell death by apoptosis occurs after B cell expansion to prevent lymphocytic hyperplasia (25). The ability of LANA2 to prevent **p53**-mediated B cell apoptosis would be an apparent benefit in maintaining an expanded population of infected cells, or in preventing **p53** pathway activation as part of a cellular antiviral response. While our in vitro studies suggest that LANA2 inhibition of **p53** activity is through direct protein-protein interaction, caution is

necessary in interpreting these results since they were not confirmable through in vivo interaction assays. The **p53** region binding LANA2 (aa. 290-393) in GST-pulldown assays includes the **p53** tetramerization and regulatory domains, as well as residues acetylated by p300 (17), suggesting a plausible mechanism.

DETD [0194] The reasons why KSHV possesses two latency-expressed viral proteins, LANA1 and LANA2, to target the same **p53** tumor suppressor protein are unclear. LANA1 is constitutively expressed in both KS lesions as well as KSHV-infected hematopoietic tissues and. . . appears to have a broader functional spectrum than LANA2. It is important to note that our LANA2 experiments showing functional **p53** inhibition were performed in osteosarcoma cell lines and so, at least under the conditions of our assays, LANA2 inhibition of **p53** is not unique to B cell lines.

DETD [0195] Regardless of the mechanism for **p53**-inhibition, LANA2 is a likely candidate protein involved in cell proliferation in hematopoietic tissues. Inhibition of **p53**-induced apoptosis may contribute to B cell hyperplasia in Castleman's disease and to cell transformation in PEL cells. Although KSHV vCYC. . . cyclin homolog has been difficult to achieve in vitro since it induces apoptosis (31). Direct inhibition of both pRB and **p53** signaling pathways by vCYC together with LANA1 and LANA2 could theoretically contribute to proliferative/neoplastic expansion of infected B cells.

DETD . . . Lengauer, T. Waldman, S. Zhou, J. P. Brown, J. M. Sedivy, K. W. Kinzler, and B. Vogelstein 1998. Requirement for **p53** and p21 to sustain G2 arrest after DNA damage. Science. 282:1497-501.

DETD [0208] 13. Friborg, J., Jr., W. Kong, M. O. Hottiger, and G. J. Nabel 1999. **p53** inhibition by the LANA protein of KSHV protects against cell death. Nature. 402:889-94.

DETD [0212] 17. Gu, W., and R. G. Roeder 1997. Activation of **p53** sequence-specific DNA binding by acetylation of the **p53** C-terminal domain. Cell. 90:595-606.

DETD . . . S. Lain, C. A. Midgley, D. P. Lane, and R. T. Hay 1999. SUMO-1 modification activates the transcriptional response of **p53**. EMBO Journal. 18:6455-6461.

CLM What is claimed is:
73. A method of inhibiting **p53** mediated apoptosis of a cell which comprises introducing into the cell an effective amount of the replicable vector of claim 13, so as to thereby inhibit **p53** mediated apoptosis of the cell.

. . . a cell which comprises introducing into the cell an amount of the replicable vector of claim 13 effective to inhibit **p53** mediated apoptosis of the cell, so as to thereby immortalize the cell.

. . . introducing into a cell which produces the antibody an amount of the replicable vector of claim 13 effective to inhibit **p53** mediated apoptosis of the cell and thereby immortalizing the cell, so as to thereby produce the antibody.

L7 ANSWER 12 OF 22 USPATFULL on STN

DETD [0085] The XhoI fragment resulting from cloning the two **exon/intron junction** fragments together can be cleaved with either BamHI or BglII, depending on which enzyme was used for excision step above, and the genomic 6.8 kb BamHI segment, containing the KPI and OX-2 coding region along with their flanking **intron** sequences, can be inserted. This fragment was identified by Kitaguchi et al. (1988) using Southern blot analysis of BamHI-digested lymphocyte. . . eight Alzheimer's disease patients using a 212 bp TaqI-AvaI fragment, nucleotides 862 to 1,073, of APP770 cDNA as the hybridization **probe**. Genomic DNA clones containing the region of the 225 bp insert can be isolated, for example, from a human leukocyte DNA library using the 212 bp TaqI-AvaI fragment as a **probe**. In the genomic

DNA, the 225 bp sequence is located in a 168 bp **exon** (**exon** 7) and a 57 bp **exon** (**exon** 8), separated by an **intron** of approximately 2.6 kb (**intron** 7), with both exons flanked by **intron-exon** consensus sequences. The **exon** 7 corresponds to nucleotides 866 to 1,033 of APP770, and the **exon** 8 to nucleotides 1,034 to 1,090. **Exon** 7 encodes the highly conserved region of the Kunitz-type protease inhibitor family domain.

DETD . . . J. Cell Biology 127:1717-1727 (1994)), cyclin D1 (Freeman et al., Neuron 12:343-355 (1994); Kranenburg et al., EMBO Journal 15:46-54 (1996)), **p53** (Chopp, Current Opinion in Neurology & Neurosurgery 6:6-10 (1993); Sakhi et al., Proc. Natl. Acad. Sci. USA 91:7525-7529 (1994); Wood. . .

CLM What is claimed is:

. . . interferon-alpha, S100.beta., cPLA.sub.2, c-jun, c-fos, HSP27, HSP70, MAP5, membrane lipid peroxidase, protein carbonyl formation, junB, jund, fosB, fra1, cyclin D1, **p53**, NGFI-A, NGFI-B, I.kappa.B, NF.kappa.B, IL-8, MCP-1, MIP-1.alpha., matrix metaloproteinases, 4-hydroxynonenal-protein conjugates, amyloid P component, laminin, and collagen type IV.

. . . interferon-alpha, S100.beta., cPLA.sub.2, c-jun, c-fos, HSP27, HSP70, MAP5, membrane lipid peroxidase, protein carbonyl formation, junB, junD, fosB, fra1, cyclin D1, **p53**, NGFI-A, NGFI-B, I.kappa.B, NF.kappa.B, IL-8, MCP-1, MIP-1.alpha., matrix metaloproteinases, 4-hydroxynonenal-protein conjugates, amyloid P component, laminin, and collagen type IV.

L7 ANSWER 13 OF 22 USPATFULL on STN

DETD A 223 base pair (bp) DNA fragment made up of 110 bases of **intron** 3 and all 113 bases of **exon** 4 of the mouse GRP78 gene was synthesized by PCR using genomic DNA as template and inserted into pT7/T3 (Ambion, Austin, Texas). Two probes of the **junction** region of **intron** 7 and **exon** 7 of the GRP78 gene were produced by PCR using mouse genomic DNA as template. A 257-base fragment including all of **exon** 7 and the first 113 bases of **intron** 7 was produced. A 200-base fragment including all of **exon** 7 and the first 56 bases of **intron** 7 also was produced. The T7 RNA polymerase promoter was ligated to these PCR fragments using a Lig'nScribe kit as. . . protection assays were performed using an RPA II kit as described by the supplier (Ambion). Hybridization of the 257-base RNA **probe** with GRP78 pre-mRNA protected all 257-bases corresponding to **exon** 7 and the first 113 bases of **intron** 7. Hybridization of the 200-base RNA **probe** to pre-mRNA protected 200-bases corresponding to all of **exon** 7 and the first 56 bases of **intron** 7. Hybridization of either **probe** to GRP78 mRNA protects the 143-bases complementary to **exon** 7. A 185- and a 277-bp cDNA fragment of S-II cDNA was synthesized and subcloned into pT7/T3 (12). [.sup.32P]-labeled RNA. . .

DETD . . . Caspase 3 (Casp3); cysteine protease mediator of apoptosis; ubiquitous; ET63241

3 4 0.005 Cyclin G (Ccng); augments apoptosis; target gene of **P53**; liver, elsewhere; Z37110

>100 >100 <0.001 Fused toes (Fts); a gene related to ubiquitin-conjugating enzymes; suggested role in apoptosis during development; expression distribution poorly defined; X71978

22 21 <0.001 **P53** specific ubiquitin ligase 2 (Mdm2); promotes ubiquitination and proteaesome degradation of **p53**; inactivation by stress causes cell cycle arrest and apoptosis; liver, elsewhere; X58876

>100 >100 <0.001 RNA-dependent EIF-2 alpha kinase; double-stranded RNA-
. . .

L7 ANSWER 14 OF 22 USPATFULL on STN
SUMM . . . is also influenced by cell type and the presence or absence of other proteins that interact with WT1, such as **p53**, the prostatic apoptosis response protein PAR-4 known as PAWR and CIOA 1 which have been shown to decrease the transcriptional. . .
DETD . . . of this WT1 gene product. Given the effect of interaction with other proteins on the biological consequence of WT1 expression (**P53**, PAR-4, CIAO 1, and Hsp 40), the protein encoded by this short transcript may contribute directly and/or indirectly to the. . .
DETD [0044] The origin of the product from WT1 was confirmed through the hybridization of each of the products with a **probe** located in **exon** 10 (Table 1). In contrast, RT-PCR was performed using primers that spanned the first splice **junction** (**exon** 1-**exon** 2), no products were detected in P69SV40TAg or any of its sublines as shown in FIG. 2. Furthermore, no products. . .
DETD . . . genes are known to exploit intronic promoters under appropriate conditions. The human mdm2 gene utilizes an intronic promoter that is **p53**-responsive. The c-kit gene uses a promoter in intron 16, which is active in a cell- and developmental-stage specific fashion. Without. . .

L7 ANSWER 15 OF 22 USPATFULL on STN
SUMM Microsatellite markers have also been used for colon cancer detection (L. Cawkwell, et. al., "Frequency of Allele Loss of DCC, **p53**, RB1, WT1, NF1, NM23, and APC/MCC in Colorectal Cancer Assayed by Fluorescent Multiplex Polymerase Chain Reaction," Br. J. Cancer 70(5):. . .
DRWD FIGS. 26A-C show electropherogram results for an LDR/PCR process of ErbB, G6PD, Int2, **p53**, and SOD gene segments from normal human female DNA and from DNA of the breast cancer cell line ZR-75-30 and. . . FAM-labeled products of 58 bp (ErbB (i.e. HER-2/neu/erbB oncogene)), 61 bp (G6PD), 67 bp (Int2 (i.e. int-2 oncogene)), 70 bp (**p53**) and 76 bp (SOD). These products are separated and analyzed on a 373A DNA sequencer using the Genescan 672 software. . . determination for the five loci in normal human female DNA is shown. The peak heights and areas for G6PD, Int2, **p53**, and SOD are very similar. The peak height and area for ErbB is consistently small in normal genomic DNA. In. . .
DRWD . . . of ErbB affected the relative peak heights of the other LDR oligonucleotide probes and PCR oligonucleotide primers for G6PD, Int2, **p53**, and SOD. In FIG. 27A, the gene dosage determination for the four loci in normal human female DNA is shown. Peak heights and areas for G6PD, Int2, **p53**, and SOD are similar, as they were in the experiment using all five LDR primers. In FIG. 27B, G6PD, Int2,. . . cancer cell line show similar relative peak heights, comparable to their appearance in normal female DNA. The peak height for **p53** is reduced, suggesting the deletion of this gene in a portion of the cells in this cell line. In FIG. 27C, in the gastric carcinoma cell line, SKGT-2, G6PD, and **p53** show comparable peak heights. The Int2 peak height remains relatively high, as it was in the experiment using all five. . .
DETD . . . of 104, 107, and 110, with the peak areas representing amplification of the Her-2 gene, loss of heterozygosity of the **p53** gene, and the control SOD gene, respectively. The electrophoresis curve where steps 3b and 4b are used involves three ligation. . . of 58, 70, and 76, with the peak areas representing amplification of the Her-2 gene, loss of heterozygosity of the **p53** gene, and the control SOD gene, respectively.
DETD . . . sequences hybridizing to gene-specific addresses, where the fluorescent intensity represents amplification of the Her-2 gene, loss of heterozygosity of the **p53** gene, and the control SOD gene,

respectively.

DETD . . . process for multiplex detection of gene amplifications and deletions. Here, the ratio of the Her-2/neu gene from Chromosome 17q, the **p53** gene from Chromosome 17p, and the SOD gene from Chromosome 21q is detected. Following denaturation of DNA at 94.degree. C., . . .

DETD . . . both alleles (i.e., chromosomes) containing RB1 and NM23 and loss of heterozygosity (i.e., loss of allele on one chromosome) for **p53**.

DETD . . . both alleles (i.e., chromosomes) containing RB1 and NM23 and loss of heterozygosity (i.e., loss of allele on one chromosome) for **p53**.

DETD . . . oncogenes, tumor suppressor genes, or genes involved in DNA amplification, replication, recombination, or repair. Examples of these include: BRCA1 gene, **p53** gene, APC gene, Her2/Neu amplification, Bcr/Ab1, K-ras gene, and human papillomavirus Types 16 and 18. Various aspects of the present. . .

DETD . . . oligonucleotide probes for quantification of gene amplifications and deletions in the LDR/PCR process. These oligonucleotide probes were designed to recognize **exon** 8 in the **p53** tumor suppressor gene (on chromosome 17p), **exon** 3 of int-2 (on chromosome 11q), an internal **exon** in HER-2/neu (i.e. HER-2/neu/erbB oncogene) (on chromosome 17q), **exon** 3 in SOD (i.e. super oxide dimutase) (on chromosome 21q), and **exon** 6 in G6PD (i.e. glucose 6-phosphate dehydrogenase) (on chromosome Xq). Each pair of LDR oligonucleotide probes has the following features: (i) The left oligonucleotide **probe** contains from 5' to 3' an 18 base sequence identical to the fluorescently labeled secondary oligonucleotide primer (black bar), an. . . target-specific sequence of from 22 to 28 bases with a T.sub.m of 75.degree. C. (patterned bar); (ii) The right oligonucleotide **probe** contains from 5n to 3' a target-specific sequence of 20-25 bases with a T.sub.m of 75.degree. C. (patterned bar), a. . . each unique restriction site generates a product which differs by at least 2 bases from the other products. Each oligonucleotide **probe** set has an **exon**-specific region chosen to ligate the **junction** sequence of (A, T)C.dwnarw.C(A, T). This **junction** sequence corresponds to either a proline residue (codon CCN) or the complementary sequence of a tryptophan residue (TGG). These sequences were chosen to minimize differences in ligation rates and the chance of a polymorphism at the ligation **junction**.

DETD In the normal female, the ErbB2 peak is lower, and the **p53** peak is slightly lower than the remaining 3 peaks. See FIGS. 25A-D. In different experiments, it was observed that the ErbB2 peak is always lower, the G6PD, Int-2, **p53**, and SOD peak areas would vary somewhat, but all 5 peaks would retain the same relative profile from one sample. . . these two cell lines. In addition, cell line NM10 appears to have undergone LOH (i.e. a loss of heterozygosity) of **p53**, while cell line SKBR3 appears to have undergone LOH of G6PD and **p53**. Some of the cells in cell line SKBR3 may have lost both copies of the **p53** gene. Repeating these amplifications in the absence of the ErbB-2 primers was used to confirm the presence of these additional. . .

DETD TABLE 3

Raw Peak Area Data

	Genes				
	ErB	G6PD	Int2	**p53**	SOD
Male	9954	21525	45688	36346	62506
Female	8340	39309	39344	30270	54665
NM10	20096	55483	67083	17364	84339
SKBR3	106650	19120	50103	2888.	. .

DETD TABLE 4

Ratio of Peak Areas to SOD Peak Area

	ErbB/SOD	G6PD/SOD	Int2/SOD	**p53**/SOD

Male	0.16	0.34	0.73	0.58
Female	0.15	0.72	0.72	0.55
NM10	0.24	0.66	0.80	0.21
SKBR3	2.22	0.40	1.04	0.06

DETD TABLE 5

Ratio of Peak Areas Ratios

	ErbB/2	G6PD	Int2	**p53**
Female/Male	0.96	2.09	0.98	0.95
NM10/Male	1.50	1.91	1.09	0.35
SKBR3/Male	13.92	1.15	1.42	0.10

DETD . . . can be determnined that the normal male and female have the same number of genes on chromosomes 17q (ErbB), 17p (**p53**), and 11q (Int 2), but that the female has twice as many G6PD genes, or X chromosomes. Likewise, cell line NM10 showed slight amplification of the ErbB-2 gene, and LOH at **p53**, while cell line SKBR3 shows significant amplification of the ErbB-2 gene, LOH at G6PD and **p53**. To confirm additional gene amplifications and deletions, primer pairs causing massive amplifications may be removed from the LDR/PCR reaction (see. . .

DETD . . . the ErbB2 peak is lower than the remaining 4 peaks. In different experiments, it was observed that the G6PD, Int-2, **p53** , and SOD peak areas would vary somewhat, but would retain the same relative profile from one sample to the next.. . . the known ErbB-2 gene amplification in these two cell lines. In addition, the ZR-75-30 line appears to show LOH of **p53**, while the SKGT-2 cell line appears to have a slight amplification of the Int-2 region. By repeating these LDR/PCR experiments. . .

DETD TABLE 6

Raw Peak Area Data

	Genes ErbB	G6PD	Int2	**p53**	SOD
Female; 4 Primer Sets	NA	9577	8581	9139	8128
ZR7530; 4 Primer Sets	NA	8452	7904	4168	7996

SKGT2; 4 Primer Sets. . .

DETD TABLE 6

Raw Peak Area Data

	Genes ErbB	G6PD	Int2	**p53**	SOD
Female; 4 Primer Sets	NA	9577	8581	9139	8128
ZR7530; 4 Primer Sets	NA	8452	7904	4168	7996

SKGT2; 4 Primer Sets. . .

DETD One can quantify the amount of ErbB2 and Int-2 amplification as well as **p53** deletion by comparing the ratio of peak area ratios between normal DNA and cancer cell lines, as shown in Table. . .

DETD TABLE 8

Ratio of Peak Area Ratios

	ErbB	G6PD	Int2	**p53**
Female; 4/5	NA	1.10	1.16	1.07
ZR7530; 4/5	NA	0.89	1.04	1.16
SKGT2; 4/5	NA	0.79	0.97	1.04

ZR7530/Female; 4/4. . .

DETD . . . and 5 primer amplifications (with the exception of SKGT2-G6PD noted above). The ZR7530 cell line. demonstrates a clear LOH for **p53**, while the SKGT2 cell line shows amplification of the Int-2 region, and both **p53** genes present.

L7 ANSWER 16 OF 22 USPATFULL on STN

SUMM . . . is also influenced by cell type and the presence or absence of other proteins that interact with WT1, such as **p53**, the prostatic apoptosis response protein PAR-4 known as PAWR and CIOA 1 which have been shown to decrease the transcriptional. . .

DETD . . . of this WT1 gene product. Given the effect of interaction with other proteins on the biological consequence of WT1 expression (**P53**, PAR-4, CIAO 1, and Hsp 40), the protein encoded by this

short transcript may contribute directly and/or indirectly to the. . .

DETD The origin of the product from WT1 was confirmed through the hybridization of each of the products with a **probe** located in **exon** 10 (Table 1). In contrast, RT-PCR was performed using primers that spanned the first splice **junction** (**exon** 1-**exon** 2), no products were detected in P69SV40TAg or any of its sublines as shown in FIG 2. Furthermore, no products. . .

DETD . . . genes are known to exploit intronic promoters under appropriate conditions. The human mdm2 gene utilizes an intronic promoter that is **p53**-responsive. The c-kit gene uses a promoter in intron 16, which is active in a cell- and developmental-stage specific fashion. Without. . .

L7 ANSWER 17 OF 22 USPATFULL on STN

SUMM Microsatellite markers have also been used for colon cancer detection (L. Cawkwell, et. al., "Frequency of Allele Loss of DCC, **p53**, RB1, WT1, NF1, NM23, and APC/MCC in Colorectal Cancer Assayed by Fluorescent Multiplex Polymerase Chain Reaction," Br. J. Cancer 70(5):. . .

DRWD FIGS. 26A-C show electropherogram results for an LDR/PCR process of ErbB, G6PD, Int2, **p53**, and SOD gene segments from normal human female DNA and from DNA of the breast cancer cell line ZR-75-30 and. . . FAM-labeled products of 58 bp (ErbB (i.e. HER-2/neu/erbB oncogene)), 61 bp (G6PD), 67 bp (Int2 (i.e. int-2 oncogene)), 70 bp (**p53**) and 76 bp (SOD). These products are separated and analyzed on a 373A DNA sequencer using the Genescan 672 software. . . determination for the five loci in normal human female DNA is shown. The peak heights and areas for G6PD, Int2, **p53**, and SOD are very similar. The peak height and area for ErbB is consistently small in normal genomic DNA. In. . .

DRWD . . . of ErbB affected the relative peak heights of the other LDR oligonucleotide probes and PCR oligonucleotide primers for G6PD, Int2, **p53**, and SOD. In FIG. 27A, the gene dosage determination for the four loci in normal human female DNA is shown. Peak heights and areas for G6PD, Int2, **p53**, and SOD are similar, as they were in the experiment using all five LDR primers. In FIG. 27B, G6PD, Int2,. . . cancer cell line show similar relative peak heights, comparable to their appearance in normal female DNA. The peak height for **p53** is reduced, suggesting the deletion of this gene in a portion of the cells in this cell line. In FIG. 27C, in the gastric carcinoma cell line, SKGT-2, G6PD, and **p53** show comparable peak heights. The Int2 peak height remains relatively high, as it was in the experiment using all five. . .

DETD . . . of 104, 107, and 110, with the peak areas representing amplification of the Her-2 gene, loss of heterozygosity of the **p53** gene, and the control SOD gene, respectively. The electrophoresis curve where steps 3b and 4b are used involves three ligation. . . of 58, 70, and 76, with the peak areas representing amplification of the Her-2 gene, loss of heterozygosity of the **p53** gene, and the control SOD gene, respectively.

DETD . . . sequences hybridizing to gene-specific addresses, where the fluorescent intensity represents amplification of the Her-2 gene, loss of heterozygosity of the **p53** gene, and the control SOD gene, respectively.

DETD . . . process for multiplex detection of gene amplifications and deletions. Here, the ratio of the Her-2/neu gene from Chromosome 17q, the **p53** gene from Chromosome 17p, and the SOD gene from Chromosome 21q is detected. Following denaturation of DNA at 94.degree. C.,. . .

DETD . . . both alleles (i.e., chromosomes) containing RB1 and NM23 and loss of heterozygosity (i.e., loss of allele on one chromosome) for **p53**.

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DETD . . . oncogenes, tumor suppressor genes, or genes involved in DNA amplification, replication, recombination, or repair. Examples of these include: BRCA1 gene, **p53** gene, APC gene, Her2/Neu amplification, Bcr/Abl, K-ras gene, and human papillomavirus Types 16 and 18. Various aspects of the present. . .

DETD . . . Xq28 G6PD (48) G6PDEx6- (48) exon 6
Ex6-3L 4R W1145
Int2 11q13 Int2 (50) Int2Ex3-8R (46) exon 3
Ex3-7L W135
p53 17p13.1 **p53** (52) p53Ex8-10R (44) exon 8
Ex8-9L P51
SOD 21q22.1 SOD (49) SODEx3- (47) exon 3
Ex3-11L 12R P355

DETD . . . oligonucleotide probes for quantification of gene amplifications and deletions in the LDR/PCR process. These oligonucleotide probes were designed to recognize **exon** 8 in the **p53** tumor suppressor gene (on chromosome 17p), **exon** 3 of int-2 (on chromosome 11q), an internal **exon** in HER-2/neu (i.e. HER-2/neu/erbB oncogene) (on chromosome 17q), **exon** 3 in SOD (i.e. super oxide dimutase) (on chromosome 21q), and **exon** 6 in G6PD (i.e. glucose 6-phosphate dehydrogenase) (on chromosome Xq). Each pair of LDR oligonucleotide probes has the following features: (i) The left oligonucleotide **probe** contains from 5' to 3' an 18 base sequence identical to the fluorescently labeled secondary oligonucleotide primer (black bar), an. . . target-specific sequence of from 22 to 28 bases with a T.sub.m of 75.degree. C. (patterned bar); (ii) The right oligonucleotide **probe** contains from 5' to 3' a target-specific sequence of 20-25 bases with a T.sub.m of 75.degree. C. (patterned bar), a. . . each unique restriction site generates a product which differs by at least 2 bases from the other products. Each oligonucleotide **probe** set has an **exon**-specific region chosen to ligate the **junction** sequence of (A, T)C.dwnarw.C(A, T). This **junction** sequence corresponds to either a proline residue (codon CCN) or the complementary sequence of a tryptophan residue (TGG). These sequences were chosen to minimize differences in ligation rates and the chance of a polymorphism at the ligation **junction**.

DETD In the normal female, the ErbB2 peak is lower, and the **p53** peak is slightly lower than the remaining 3 peaks. See FIGS. 25A-D. In different experiments, it was observed that the ErbB2 peak is always lower, the G6PD, Int-2, **p53**, and SOD peak areas would vary somewhat, but all 5 peaks would retain the same relative profile from one sample. . . these two cell lines. In addition, cell line NM10 appears to have undergone LOH (i.e. a loss of heterozygosity) of **p53**, while cell line SKBR3 appears to have undergone LOH of G6PD and **p53**. Some of the cells in cell line SKBR3 may have lost both copies of the **p53** gene. Repeating these amplifications in the absence of the ErbB-2 primers was used to confirm the presence of these additional. . .

DETD TABLE 3

Raw Peak Area Data

	Genes				
	ErB	G6PD	Int2	**p53**	SOD
Male	9954	21525	45688	36346	62506
Female	8340	39309	39344	30270	54665
NM10	20096	55483	67083	17364	84339
SKBR3	106650. . .				

DETD TABLE 4

Ratio of Peak Areas to SOD Peak Area

	ErbB/SOD	G6PD/SOD	Int2/SOD	**p53**/SOD
Male	0.16	0.34	0.73	0.58
Female	0.15	0.72	0.72	0.55
NM1O	0.24	0.66	0.80	0.21
SKBR3	2.22	0.40	1.04	0.06

DETD TABLE 5

Ratio of Peak Areas Ratios	ErbB/2	G6PD	Int2	**p53**
Female/Male	0.96	2.09	0.98	0.95
NM10/Male	1.50	1.91	1.09	0.35
SKBR3/Male	13.92	1.15	1.42	0.10

DETD . . . can be determined that the normal male and female have the same number of genes on chromosomes 17q (ErbB), 17p (**p53**), and 11 q (Int 2), but that the female has twice as many G6PD genes, or X chromosomes. Likewise, cell line NM10 showed slight amplification of the ErbB-2 gene, and LOH at **p53**, while cell line SKBR3 shows significant amplification of the ErbB-2 gene, LOH at G6PD and **p53**. To confirm additional gene amplifications and deletions, primer pairs causing massive amplifications may be removed from the LDR/PCR reaction (see. . .

DETD . . . the ErbB2 peak is lower than the remaining 4 peaks. In different experiments, it was observed that the G6PD, Int-2, **p53** , and SOD peak areas would vary somewhat, but would retain the same relative profile from one sample to the next.. . . the known ErbB-2 gene amplification in these two cell lines. In addition, the ZR-75-30 line appears to show LOH of **p53**, while the SKGT-2 cell line appears to have a slight amplification of the Int-2 region. By repeating these LDR/PCR experiments. . .

DETD TABLE 6

Raw Peak Area Data	Genes ErbB	G6PD	Int2	**p53**	SOD
Female; 4 Primer Sets	NA	9577	8581	9139	8128
ZR7530; 4 Primer Sets	NA	8452	7904	4168	7996
SKGT2; 4. . .					

DETD TABLE 7

Ratio of Peak Areas to SOD Peak Area	ErbB/SOD	G6PD/SOD	Int2/SOD	**p53**/SOD
Female; 4 Primer Sets	NA	1.18	1.06	1.12
ZR7530; 4 Primer Sets	NA	1.06	0.99	0.52
SKGT2; 4 Primer Sets NA. . .				

DETD One can quantify the amount of ErbB2 and Int-2 amplification as well as **p53** deletion by comparing the ratio of peak area ratios between normal DNA and cancer cell lines, as shown in Table. . .

DETD TABLE 8

Ratio of Peak Area Ratios	ErbB	G6PD	Int2	**p53**
Female; 4/5	NA	1.10	1.16	1.07

ZR7530; 4/5 NA 0.89 1.04 1.16
SKGT2; 4/5 NA 0.79 0.97 1.04
ZR7530/Female; 4/4 NA. . .

DETD . . . and 5 primer amplifications (with the exception of SKGT2-G6PD noted above). The ZR7530 cell line demonstrates a clear LOH for **p53**, while the SKGT2 cell line shows amplification of the Int-2 region, and both **p53** genes present.

L7 ANSWER 18 OF 22 USPATFULL on STN

SUMM Another important example of a tumor suppressor gene is the **p53** TSG, whose biological activity has been elucidated in-vitro through molecular and biochemical studies before it became identified as the genetic. . .

SUMM . . . using an in-vitro functional expression transductory cloning technique. The described novel class of tumor suppressor proteins shares the ability of **p53** to inhibit growth of tumor cells by controlling apoptotic cell death and cell cycle progression and appears to play a. . . newly identified tumor suppressors display a restricted pattern of tissue expression and distinct activities compared to known TSGs such as **p53**.

SUMM . . . well as mechanisms unidentified so far. "Tumor suppressors" are proteins displaying biological activities identical to or similar to those of **p53**, Rb (retinoblastoma gene product), WT (Wilms tumor suppressor gene), VHL (von Hippel-Lindau tumor suppressor gene), BRCA1 (breast cancer susceptibility gene). . .

SUMM . . . Thus, the protein of the invention displays all essential features of a tumor suppressor similar to those of, for example, **p53**. This new tumor suppressor is also able to induce apoptosis resulting in inhibition of tumor cell growth. However, this new tumor suppressor exhibits functional differences compared to **p53**, for instance the induction of apoptotic cell death is more pronounced in Saos-2 cells for the protein of the invention than for **p53**. Furthermore, the tumor suppressor of the invention induces G1 arrest of the cell cycle, in contrast to **p53**, independently from the transactivation of the gene encoding the cyclin-dependent kinase inhibitor p21. Finally, it had been shown that this. . .

SUMM . . . be those which code for proteins in which putative phosphorylation sites are altered. Biochemical analysis of the regulation of wild-type **p53** sequence-specific DNA binding has, for instance, shown that the unphosphorylated tetramer has a cryptic sequence-specific DNA binding activity. This cryptic or latent state of **p53** depends upon a C-terminal negative regulatory domain, which locks the unphosphorylated tetramer in an inactive state. Phosphorylation of the C-terminal negative regulatory domain of latent **p53** by either protein kinase C or casein kinase II or deletion of the regulatory domain activates sequence-specific DNA binding. In addition, a monoclonal antibody can mimic the effects of protein kinases and activate latent **p53**. Thus, neutralization of this negative regulatory domain by covalent or non-covalent modification is an important stage in the activation of **p53**. As described above, the protein encoded by SEQ ID NO. 1 has two putative phosphorylation sites for protein kinases. A. . .

SUMM . . . Mice deficient for Rb revealed massive neuronal cell death due to the failure to stop cell division. A subset of **p53** -deficient mice (8 to 16%) exhibit exencephaly and a large population (40%) of Brca1-deficient mice embryos suffered to varying degrees of. . .

SUMM Furthermore, recent reports indicated that **p53**-dependent apoptosis modulates the cytotoxic effects of common antitumor agents such as ionizing radiation, fluorouracil, etoposide, and doxrubicin. Cells lacking wild-type **p53** are resistant to these agents, whereas cells expressing wild-type **p53** are sensitive to them and undergo cell death by apoptosis. These observations raise the exciting prospect that **p53** mutations may provide a genetic

basis for drug resistance. In the presence of **p53**, oncogene-expressing cells can form tumors, but cell survival is limited by their increased susceptibility to apoptosis. Conversely, **p53** loss contributes directly to immortalization and tumorgenesis, probably by abrogating an intrinsic senescence program. As a consequence, selection against **p53** often occurs late in tumor progression. Anticancer agents may simply activate the apoptotic program intrinsic to these sensitized cells. These. . .

SUMM . . . according to the invention caused apoptotic cell death in transformed cell lines, which in part exceeded the one caused by **p53**, these novel TSGs present a powerful option of high potential interest in gene therapy experiments. Though **p53** and the protein encoded by SEQ ID NO. 1 induce at a descriptive level the same responses, namely cell-cycle regulation. . . ID NO. 1 is organized in a typical zinc finger structure, which is unrelated to the central DNA-binding domain of **p53**. Therefore, the protein encoded by SEQ ID NO. 1 and related proteins could replace **p53** in gene therapy strategies. Importantly **p53** seems only to trigger growth arrest and not cell death in some cell types and under some conditions. In line with this view we demonstrated that restoration of inducible **p53** function in the **p53**-negative cell line Saos-2 (human, osteosarcoma) installed preferentially a growth and a comparatively weak apoptotic response, whereas Saos-2 cells became highly. . . ID NO. 1. This differential apoptotic response emphasizes the idea that this protein and other TSGs of the invention and **p53** supply different molecular routes to apoptosis and open the exciting perspective that apoptosis competency is a tissue-specific encoded genetic program.. . . could encode specific properties to guide tumorigenic cells to apoptotic cell death and their potency to do so could surpass **p53** as illustrated for the protein encoded by SEQ ID NO. 1 in Saos-2 cells.

SUMM Importantly again, the understanding of **p53** function as an example for a tumor suppressor gene suggest a basis for the association between **p53** mutations and poor patient prognosis. Thus, **p53** mutations, which are with 50% among the most common alterations observed in human cancer, may be a significant impediment to successful cancer therapy. For example, **p53** mutations dramatically reduce the probability that patients with B cell chronic lymphocyte leukemia will enter remission after chemotherapy. Similarly evaluation. . .

SUMM Some genetic changes lead to altered protein conformational states. For example, mutant **p53** proteins possess a tertiary structure that renders them far less capable of binding to their wild-type DNA recognition elements. Restoring. . . NO. 1 are expressed in a tissue-specific manner deserves particular attention. All pharmacological manipulations aimed at restoration of wild-type conformation **p53**, bear the risk to interfere with the wild-type function of this tumor suppressor in neighboring non-tumorgenic tissues with profound side-effects.. . .

DRWD FIG. 2A-2D: Bop1 and **p53** Alter Proliferation of LLC-PK1 and Saos-2 Cells

DRWD Anhydrotetracycline(ATc)-regulated expression of Bop1 and **p53** was established in LLC-PK1 and Saos-2 cells.

DRWD (A) Cell counts of the parent tTA clones (L-tTA and S-tTA) in comparison to Bop1- and **p53**-expressing LLC-PK1 (L-Bop and L-**p53**, respectively) and Saos-2 (S-Bop and S-**p53**, respectively) clones in the presence (+) and absence (-) of ATc.

DRWD (B) Bop1 and **p53** inhibit DNA-synthesis (BrdU) and cell viability (MTT). For each time point, BrdU incorporation or formazan blue formation were measured in. . .

DRWD (C) Growth inhibition by Bop1 and **p53** is serum independent. Cells were grown in the presence of the indicated amount of fetal bovine serum (10% or 0.1%). . .

DRWD (D) Growth inhibition by Bop1 and **p53** is reversible. Cells

were seeded in Atc-containing medium, grown in the absence of ATc for 2 days before medium was. . .

DRWD FIGS. 3A-3D: Bop1 and **p53** Inhibit Soft Agar Colony Formation

DRWD Bop1 (L-Bop and S-Bop) and **p53** (L-**p53** and S-**p53**) clones were grown in the presence of ATc before plating into soft agar at densities of 1.times.10.sup.5 (No. 1+4), 5.times.10.sup.4. . .

DRWD FIG. 4A-4C: Bop1 and **p53** Induce Apoptotic Cell Death

DRWD (A) DNA laddering. Genomic DNA was isolated from Bop1 (L-Bop and S-Bop) and **p53** (L-**p53** and S-**p53**) expressing clones grown in the presence (+) or absence (-) of ATc for 3 days, centrifugated and soluble DNA was. . .

DRWD (B) Fluorescence microscopy of Bop1 and **p53** clones stained with ethidium bromide and acridine orange. Cells (a: L-Bop; b: L-**p53**; c: S-Bop; d: S-**p53**) were grown in the absence of ATc for 3 days. Floating cells were collected, incubated with ethidium bromide and examined. . .

DRWD (C) DNA end labeling. S-Bop (Bop1) and S-**p53** (**p53**) cells were grown for 3 days in the presence (black) or absence (grey) of ATc. Permeabilized cells were subjected to. . .

DRWD FIG. 5A-5C: Bop1 and **p53** Regulate Cell Cycle Distribution

DRWD (A) Induction of G1 arrest by Bop1 and G2/M arrest by **p53**. S-Bop (upper panels) and S-**p53** (lower panels) were grown in the presence (left) or absence (right) of ATc for 3 days. Propidium iodide-stained cells were. . . increased cell population in G1 from 44.7% for the repressed state to 63.0% for the expressed state of S-Bop. For **p53** a decrease in G1 and S phase from 39.4% to 31.8% and from 43.7% to 35.0% was observed, which was. . .

DRWD (B) G1-Arrest by Bop1 is independent of p21.sup.Waf1 expression. S-tTA (tTA), S-**p53** (**p53**) and S-Bop(Bop1) cells were grown in the presence (+) or absence (-) of ATc for 3 days. Western blots of total cell lysates were performed with anti-p21, anti-**p53** and anti-GST-Bop1.DELTA.ZF antisera.

DRWD (C) Apoptotic cell death following Bop1 and **p53** expression is unrelated to the cell cycle. TUNEL was carried out on permeabilized S-Bop (Bop1, upper panels) and S-**p53** (**p53**, lower panels) cells grown in the presence (left) or absence (right) of ATc for 3 days. Subsequent staining with propidium. . . presence of ATc represent less than 5% of the cells in the case of S-Bop and less than 1% for S-**p53**. In the absence of ATc, 70% of S-Bop (65% of S-**p53** resp.) cells displayed enhanced or high TUNEL fluorescence.

DRWD (B) The zinc finger domain of Bop1 confers regulation of the PVR1 gene. Native Bop1 and **p53** (left) or the hybrid GB.sub.Z M (right) cDNAs were co-transfected with the cAMP-responsive reporter p.DELTA.MC16LUC into LLC-PK1 cells (2.times.10.sup.6) and. . .

DETD . . . pRK8, a modified pRK5 vector (Spengler et al., Nature 365 (1993), 170-175). Screening of .about.0.5.times.10.sup.6 clones with the p2195 cDNA **probe** allowed the isolation of one full-length cDNA clone designated B-16, which contained a 3.7 kb insert. Transfection of B-16 into. . . 658 by a 630 bp insertion. The sequences at the boundaries of this insertion are in excellent agreement with consensus **exon-intron junction** sequences and preserve the reading frame (FIG. 1B). We observed this insertion at exactly the same position in clone p1270. . . library (FIG. 1B). This finding argues against a cloning artefact in clone B-16 and suggests the presence of an unspliced **intron** region. In support of this hypothesis, a PCR-based fragment encoding the **intron** region failed to hybridize to a poly-A.sup.+ blot from AtT-20 cells (data not shown). The distribution of Bop1 was assessed. . .

DETD Constitutive Expression of Bop1 and **p53** Abates Growth of Tumor Cells

DETD . . . to establish a Bop1 -expressing cell clone. To evaluate the possibility that Bop1 inhibits tumor growth we subcloned Bop1 and **p53** in sense and anti-sense orientation downstream of a

cytomegalovirus promoter in a vector (pCMVPUR) carrying the puromycin resistance gene.

DETD . . . addition into the human osteosarcoma cell line Saos-2 (ATCC HTB 85), which was previously shown to be growth-inhibited by wild-type **p53** (Diller et al., Mol. Cell. Biol. 10 (1990), 5772-5781). pGEM4 replaced pCMVPUR in mock transfected cells. Three electroporations for each. . . that introduction of Bop1 sense expression vectors resulted in a substantial suppression of colony formation equivalent to that induced by **p53**. Abrogation of cell growth by Bop1 or **p53** was more prominent in the Saos-2 cell line. In addition the clones that did appear after transfection of Bop1 or **p53** sense constructs into the LLC-PK1 cell line died when reexposed to selection after passaging and grew at a slow rate. . .

DETD TABLE I

Bop1 and **p53** Suppress the Growth of Tumor Cells

Cell type	(n)	plasmid	antisense		sense	ratio
LLC-PK1	3	Bop1	1014	.+-.	170	
					2	507
	3	**p53**	1452	.+-.	258	
					2	726
	1	vector	1653	.+-.	270	
	1	mock	0			
Saos-2	3	Bop1	2538	.+-.	354	
					1	2500
	3	**p53**	3779	.+-.	566	
					1	3800
	1	vector	4517	.+-.	641	
	1	mock	0			

DETD . . . Saos-2 were electrotransfected (n=3) with the parent vector pCMVPUR or with vectors encoding sense and antisense Bop1 or wild-type rat **p53**. pGEM4 carrier DNA replaced pCMVPUR in mock transfected cells. 24 hr later, cells were grown in the presence of 5. . .

DETD Bop1 and **p53** Suppress Growth of Tumor Cells

DETD . . . downstream the .DELTA.MtetO sequences via the unique Not I site. For stable transfections the plasmids p3'SStTA, PMtetO.sub.5 Bop1 and PMtetO.sub.5 **p53** were linearized with Eam1105I and 1 .mu.g of DNA was co-transfected with 3 .mu.g pGEM4 filling DNA into 2.times.10.sup.6 cells.. . . of 700 .mu.g/ml and 500 .mu.g/ml in LLC-PK1 and SaOs-2 cells, respectively. Selection for clones expressing the Bop1 gene or **p53** was carried out at a concentration of 5.0 .mu.g/ml puromycin. The following numbers of clones were screened: L-tTA: Bop1=95, **p53**=92 and S-tTA: Bop1 n=77, **p53**: n=72. All the clones revealed impaired cell growth to varying degrees under the activated state (-ATc), which was microscopically scored. . .

DETD . . . subjected to a preliminary analysis of counts of cell numbers (data not shown). The LLC-PK1- and Saos-2-derived clones (L-Bop and L-**p53**, S-Bop and S-**p53**, resp.) displaying the greatest differences in growth were further analyzed (FIG. 2A). Importantly, no major differences in the growth behavior were observed in the presence of the repressor ATc between Bop1--and **p53**-expressing clones and the parent clones L-tTA and S-tTA (FIG. 2A). Therefore the differences in cell counts on day six were. . . absence of the repressor. Measurement of proliferation rate revealed that Bop1 (L-Bop: 11-fold; S-Bop: 20-fold) was slightly less potent than **p53** (L-**p53**: 15-fold; S-**p53**: 25-fold) in reducing the growth rate of both cell lines. Western blot analysis proved that Bop1 protein was not detectable. . . noted in the activated state (data not shown

and FIG. 5B). Similar results were also obtained for the regulation of **p53** in Saos-2 and LLC-PK1 cells (data not shown and FIG. 5B). These results emphasize that the modified expression vector combines. . .

DETD . . . necessarily discriminate between alteration of cell proliferation and viability. It was therefore decided to evaluate the effects of Bop1 and **p53** expression by two complementary methods. First, DNA-synthesis was studied with a non-radioactive immunoassay based on incorporation of 2-bromodeoxyuridine (BrdU) into. . .

DETD The results obtained for S-Bob and S-**p53** emphasize the observed differences in cell counts (FIG. 2A), which correlate with those obtained in overall cell proliferation and overall viability measurements (FIG. 2B). Similar results were obtained for L-Bop and L-**p53** (data not shown). Cells from LLC-PK1 (data not shown) and Saos-2 clones kept under low serum conditions (0.1% FCS) in. . . from day three on, indicating serum-dependence to maintain logarithmic growth (FIG. 2C). In contrast, proliferation under expression of Bop1 and **p53** remained unchanged (FIG. 2C). Therefore, inhibition of tumor growth by Bop1 and **p53** proceeds through mechanisms unrelated to the presence of serum factors in these cellular models.

DETD . . . growth pattern following reexposure to ATc of the surviving cells was tested. The impairment of cell growth by Bop1 and **p53** expression was transient for both the LLC-PK1 (data not shown) and Saos-2 clones studied. Reexposure to the repressor ATc caused cells to resume logarithmic growth after 48 hr (FIG. 2D). Therefore, Bop1- and **p53**-induced changes in cell growth were not permanent and at least in part reversible, arguing against a non-specific effect of protein. . .

DETD Bop1 and **p53** Inhibit Soft-Agar Colony Formation

DETD . . . often correlated with tumorigenesis and is a strong criteria for cultured cell transformation. To test the influence of Bop1 or **p53** on anchorage-independent growth, LLC-PK1 and Saos-2 cell clones were assayed for their ability to grow in soft-agar. Each well (35-mm). . . mg/ml) and incubated for an additional 4 hr, washed once with PBS and then photographed. Colony formation by Bop1 or **p53** expressing cells (-) was dramatically reduced compared to the repressed state (+) (FIG. 3). Also the few colonies formed under Bop1 or **p53** expression were of smaller size. These results demonstrate that Bop1 and **p53** can abate anchorage-independent growth of tumor cells, one of the hallmarks of tumorigenicity.

DETD Bop1 and **p53** Suppress Tumor Formation in Nude Mice

DETD . . . placebo pellets (Innovative Research of America). Two days latter, each animal was injected subcutaneously on each side with S-Bop or S-**p53** cells which were grown in the presence of ATc, trypsinized and resuspended in PBS at a density of 5.times.10.sup.7 cells/ml.. . . presence of ATc. Two groups were injected with S-Bop cells from two independent trypsinizations whereas one experiment was performed with S-**p53** cells. Due to the clonal origin of S-Bop and S-**p53**, differences in the tumorigenicity of each clone were observed as inferred from the difference in the observed lag in tumor formation which was assessed at 11 weeks after cell injection for S-Bop and at 16 weeks for S-**p53**. S-Bop- and S-**p53** -injected animals were sacrificed at 11 and 16 weeks, respectively, dissected and the tumors were weighed. Table II presents results from two experiments with S-Bop (Bop1) and one experiment with S-**p53** (**p53**). In agreement with previous results (Chen et al., Science 250 (1990) 1576-1580), **p53** expression impaired tumor formation by Saos-2 cells in-vivo. Interestingly, Bop1 was as efficient as **p53** in inhibiting tumor formation as deduced from tumor incidence (Table II) and from the average tumor weight (193.+-.13 mg (n=14) for Tc vs. 18.+-.7 mg (n=2) for placebo). Conclusively, Bop1 and **p53** are equipotent at inhibiting tumor formation in-vivo.

DETD TABLE II

Bop1 and **p53** Inhibit Tumor Formation in-vivo

clone	tumor incidence (No. of tumor-bearing injection sites/ total No. of injection sites) placebo	Tc
S-Bop (Bop1) exp. n.sup.o 1	2/12	14/14
S-Bop (Bop1) exp. n.sup.o 2	1/12	12/12
S-**p53** (**p53**)	1/12	10/12

DETD . . . into each side of each animal, and tumor formation was scored at 11 weeks for S-Bop (Bop1) and 16 weeks (**p53**).

DETD Expression of Bop1 and **p53** Induce Apoptosis

DETD Two days following induction of **p53** expression, Saos-2 cells flattened and greatly enlarged (three to eight fold) in average diameter, which was most evident when grown. . . . in small clusters. Similar changes, though less prominent (two to fourfold increases in the average diameter), were also observed for L-**p53** (data not shown). In contrast, Bop1 expressing LLC-PK1 or Saos-2 clones appeared indistinguishable from the parent cell lines giving a first hint of functional differences between Bop1 and **p53**. Yet, an increasing number of cells with signs of lost cell viability was observed from day two onwards following Bop1 or **p53** expression. These cells failed to convert MTT, shrank in size, were abundant in phase contrast microscopy, revealed membrane blebbing, and. . . further up before detaching from the plates. For Bop1 these alterations were most evident in Saos-2 cells (S-Bop) and for **p53** in LLC-PK1 cells (L-**p53**) (data not shown) and appear reminiscent of an apoptotic cell death. This form of cell death is often accompanied by. . .

DETD Since the flattened and enlarged cell shape of **p53**-expressing cells enhanced attachment to the plastic surface, a comparable large population of cells exhibited nuclear signs of apoptosis, whereas Bop1-expressing. . .

DETD . . . indicate that the proportion of cells displaying nuclear damage was as high as 60-70% following expression of either Bop1 or **p53** .

DETD Taken together these experiments give convincing evidence that Bop1 and **p53** recruit apoptotic programs to inhibit growth of tumor cells and Saos-2 cells seem highly apoptosis proficient following expression of Bop1.

DETD Expression of Bop1 and **p53** Induces Changes in Cell Cycle Distribution

DETD . . . the mechanisms by which Bop1 might regulate cell growth the distribution of cell cycle phases was studied. Increases in wt **p53** levels are known to suppress cell growth by blocking the cell cycle at the G1 to S transition (Hunter and Pines, Cell 79 (1994), 573-582; Sherr and Roberts, Genes and Dev. 91 (1995), 1149-1163). More recently **p53** has been suggested to address an additional checkpoint by arresting cells at the G2/M boundary (Agarwal et al., Proc. Natl.. . .

DETD The results obtained for **p53** expression in the S-**p53** cell clone are in agreement with those obtained recently with a temperature-sensitive mutant **p53** in Saos-2 cells (Yamato et al., Oncogene 11 (1995), 1-6). A decrease in G1 and S phase from 39.4% to. . . 43.7% to 35.0% was observed and a clear increase in G2/M from 16.9% to 33.2%. (FIG. 5A). The failure of **p53** to proceed to a G1 arrest reflects most likely the presence of the deleted non-functional retinoblastoma gene product (Rb) in. . .

DETD . . . extended to the LLC-PK1 cell line and though shifts of populations in cell cycle phases under expression of Bop1 and

p53 were less prominent than in the Saos-2 cell clones, there was again a clear increase in G1 phase populations for. . . Bop1 (G1 59.1% vs. 43.7%; S 28.2% vs. 38.9%; G2/M 12.7% vs. 17.4%) and a shift for G2/M populations under **p53** (G1: 39.3% vs. 44.1%; S: 32.1% vs. 40.2%; G2/M: 28.6% vs. 15.7%) (data not shown).

DETD **p53** achieves G1 arrest through transactivation of the gene encoding the cyclin-dependent kinase inhibitor p21 (also designated Cip1, Waf1, Sdi1, Cap20).. . . (1991), 293-302; Weinberg, Cell 81 (1995), 323-330). The question arose whether Bop1-induced G1 arrest utilizes the same molecular pathway as **p53**. Expression of **p53** in Saos-2 cells resulted in a strong induction of the p21 protein proving an intact and efficient transactivation of the endogenous gene by the exogenous **p53** protein (FIG. 5B). Yet, no regulation of the p21 gene in Saos-2 cells was encountered following expression of Bop1 (FIG. 5B). The same results were obtained in the LLC-PK1-clones with a strong induction of p21 by **p53** (data not shown). Conclusively, Bop1 induces G1 arrest in these cellular models through molecular relays independent of p21.

DETD In a number of cellular systems, wt **p53** activation has been shown to confer growth arrest (Mercer et. al., Proc. Natl. Acad. Sci. USA 87 (1990), 6166-6170; Merlo. . . et al., Cell 62 (1990), 671-680; Roemer and Friedmann, Proc. Natl. Acad. Sci. USA 90 (1993), 9252-9256). In contrast, wt **p53** failed to cause a measurable arrest in M1 cells and cell cycle progression proceeded while viability was lost within 48. . . 352 (1993) 345-347). In that system, cells in G1 appeared to be preferentially susceptible to the death-inducing activity of wt **p53**. Therefore the question arose whether in the used cellular models, in which Bop1 and **p53** play a dual role in regulation of apoptotic cell death and cell cycle progression, a particular phase of the cycle. . . apoptotic cells. It was concluded that cell cycle arrest is not a prerequisite to apoptosis and that both Bop1 and **p53** induced apoptosis through a pathway which is independent of the one involved in cell cycle arrest.

DETD . . . to dissect functional domains of Bop1. A bimodal regulation of the PVR1 gene was observed, indistinguishable for Bop1 and wt **p53** cDNAs as measured by induction of the cAMP-sensitive luciferase gene (FIG. 6B). The decrease in PVR1 expression with high amounts. . .

DETD . . . PVR.sub.1 gene for increasing amounts of GB.sub.Z M was consistently observed, which closely paralleled the one observed for Bop1 and **p53**. In contrast the construct .DELTA.B.sub.Z M, which lacks the glucocorticoid receptor transactivation domain failed to confer regulation of PVR1, implicating. . .

DETD . . . blots were performed on total cell lysates (50 .mu.g) with the above-mentioned purified IgG or with commercially available antibodies to **p53** (Pharmingen, San Diego, USA catalog #14091A), p21.sup.Waf1 (Transduction laboratories, Lexington, USA, catalog #C24420), p27.sup.Kip1 (Transduction laboratories, catalog #K25020) and p16.sup.ink4. . .

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DETD Using a WT1 cDNA **probe**, rearranged bands were detected comigrating with the rearranged EWS bands in multiple enzyme digests in cases 1, 3, and 5.. . . expression is tissue- and developmental stage-specific (Call et al., 1990). The strong expression of a transcript hybridizing with a WT1 **probe** in DSRCT may thus be considered significant in itself; in addition, the transcript appears smaller than the known splice variants. . . in DSRCT follows the same pattern was confirmed in at least two of our cases by RT-PCR using an EWS **exon** 7 primer and WT1 **exon** 8 or 9 primers which revealed a single PCR product of the same size in both cases. Sequencing of the PCR product showed an in-frame **junction** of EWS **exon** 7 to WT1 exon 85. Thus, this chimeric RNA encodes a putative protein in which the RNA-binding domain of EWS is replaced by

the. . .

DETD Isolation and characterization of a EWS-WT1 genomic DNA **junction** fragment from DSRCT. Further to experiments in EXAMPLE 1 wherein nongermline DNA fragments were identified in multiple restriction enzyme digests. . . cloning due to its relatively large size and clear separation from germline EWS-containing BamHI fragments (FIG. 5A). A EWS cDNA **probe** was used for library screening and identified a clone containing the expected size DNA fragment. The insert fragment hybridized to. . . specific probes and comparison of restriction map data indicated a fusion of the two genes with expected breakpoints within the **intron** between exons 7 and 8 of EWS and the **intron** between exons 7 and 8 of WT1 (FIG. 5B). Sequencing using the cloned fragment as template and primers directed to EWS **exon** 8 (primers EWS 8.1 and EWS 8.2, Table 2) and WT1 **exon** 7 (primers WT1 7.1 and WT1 7.2, Table 2) showed that both exons were intact without mutation of the coding. . . EWS on chromosome 22. Although cytogenetic analysis was not performed in the tumor from which this DNA was isolated, this **junction** fragment is expected to correspond to the derivative chromosome 11 of the DSRCT-associated t(11;22) because of the centromere 5'-3' telomere. . . WT1. Detailed restriction mapping and sequencing indicate that the breakpoints are approximately 3.5 kb from the 3' end of WT1 **exon** 7 and less than 1 kb from the 5'end of EWS **exon** 8. The EWS breakpoint site identified in this DSRCT **junction** fragment is located within a region commonly involved by other EWS-related tumor specific chromosomal translocations (Zucman et al., 1993). Consistent. . .

DETD . . . common mechanism of WT1 functional alteration in Wilms' tumors. The WT1 gene product has also been shown to interact with **p53**, a tumor suppressor gene that is frequently deleted and mutated in a variety of tumors (Maheswaran et al., 1993). This interaction modulates the function of both proteins such that in the presence of wild-type **p53**, WT1 acts as a transcriptional repressor while in the absence of wild-type **p53**, WT1 is a potent transcriptional activator. These lines of evidence suggest that transcriptional activation of WT1 target genes can contribute. . .

L7 ANSWER 20 OF 22 USPATFULL on STN

SUMM . . . including gene Rb of retinoblastoma [see Friend, S. H., et al., Proc. Natl. Acad. Sci., USA, 84, 9095 (1987)], gene **p53** of colon cancer [see Lane, D. P., et al., Nature, 278, 261 (1979)] and gene WT of Wilms' tumor [see. . .

SUMM . . . method [see Science, 196, 180 (1977)] with the use of the RT-PCR product obtained by the above-mentioned method as a **probe** . The transformant thus cloned contains a cDNA which codes for the full amino acid sequence of human prohibitin or a. . . genomic clone is isolated by screening a human chromosome cosmid library with the use of the above-mentioned cDNA as a **probe**, and then the base sequences of these cDNAs are compared with those of the genomic clones the structures of which have been already determined. Thereby, the **intron**-exon junction can be analyzed.

L7 ANSWER 21 OF 22 USPATFULL on STN

SUMM . . . including gene Rb of retinoblastoma [see Friend, S. H., et al., Proc. Natl. Acad. Sci., U.S.A., 84, 9095 (1987)], gene **p53** of colon cancer [see Lane, D. P., et al., Nature, 278, 261 (1979)] and gene WT of Wilms' tumor [see. . .

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intron-**exon** **junction** can be analyzed.

L7 ANSWER 22 OF 22 USPATFULL on STN

SUMM . . . including gene Rb of retinoblastoma [see Friend, S. H., et al., Proc. Natl. Acad. Sci., USA, 84, 9095 (1987)], gene **p53** of colon cancer [see Lane, D. P., et al., Nature, 278, 261 (1979)] and gene WT of Wilms' tumor [see. . .

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